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THE ^{13}C-N.M.R. SPECTRA OF DISACCHARIDES OF D-GLUCOSE, D-GALACTOSE, AND L-RHAMNOSE AS MODELS FOR IMMUNOLOGICAL POLYSACCHARIDES

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ABSTRACT

Complete assignments of the ^{13}C-n.m.r. spectra of disaccharides having β-glycosidic linkages are presented and discussed. The disaccharides of D-glucose, D-galactose, L-rhamnose, 2-acetamido-2-deoxy-D-glucose, and 2-acetamido-2-deoxy-D-galactose are model compounds for ^{13}C-n.m.r. studies of immunological polysaccharides. Changing the nature of the reducing glucopyranose rings (D-glucose to L-rhamnose) has no important influence on the chemical shifts of the carbons of the non-reducing glucopyranose ring (D-glucose). The converse is also true: the chemical shifts of the carbons of the reducing glucopyranose ring (L-rhamnose) are not noticeably affected by a change of the non-reducing unit (D-glucose to D-galactose or 2-acetamido-2-deoxy-D-glucose).

INTRODUCTION

^{13}C-N.m.r. spectroscopy is becoming increasingly useful for the elucidation of the composition, sequence, and conformation of polysaccharides[1–18]. The method depends entirely on the availability of the relevant model compounds. Many polysaccharides of immunological significance are composed mainly of D-glucose (or 2-acetamido-2-deoxy-D-glucose), D-galactose (or 2-acetamido-2-deoxy-D-galactose), and L-rhamnose residues with a wide variety of possible attachments[19]. As a prelude to their study, we report here the ^{13}C-n.m.r. spectra of model compounds, *i.e.*, D-glucopyranosyl-L-rhamnopyranose and D-galactopyranosyl-L-rhamnopyranose disaccharides having various types of linkage, as well as those of some disaccharides containing 2-acetamido-2-deoxy-D-glucose or 2-acetamido-2-deoxy-D-galactose.

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EXPERIMENTAL

The ^{13}C-n.m.r. spectra of the disaccharides were obtained on Varian XL-100 (25 MHz) and CFT-20 (20 MHz) spectrometers, using tubes of outside diameter 12 and 10 mm, respectively. The spectra were recorded with complete proton-decoupling, spectral windows of 200 p.p.m., and digitization into 4 K data points. Chemical shifts are accurate to ± 0.1 p.p.m. The solvent deuterium resonance was used as a field-frequency lock, and chemical shifts are expressed relative to Me_4Si contained in a coaxial sample tube of outside diameter 5 mm. Our chemical shift values differ by ~ 1 p.p.m. from those reported using indirect referencing[1,20] to Me_4Si. Samples were equilibrated in neutral deuterium oxide, 50–75 mg/ml, and spectra were taken at 32° and 30° in Varian XL-100 and CFT-20 spectrometers, respectively. D-Glucose, D-galactose, and L-rhamnose were commercial samples used without further purification. The disaccharides were synthesized as described elsewhere[21–24].

RESULTS AND DISCUSSION

Glucose–rhamnose series

The assignments of the constituent units of this series, *i.e.*, D-glucose and L-rhamnose, were previously given by several authors[1,6,25–33]. Our assignments are reported in Table I, as well as those of the disaccharides of this series. We have already compared our D-glucose assignments with those of earlier studies[6,30]. We agree with the literature values for the ^{13}C-n.m.r. spectrum of L-rhamnose[27,31]. As in previous papers[6,30], the primed carbons refer to the non-reducing unit of the disaccharide. In order to determine the configuration of the glycosidic linkage, we shall use the observation that the C-1′ of the non-reducing unit involved in a β-linkage has a larger chemical shift (resonates at a lower magnetic field) than one involved in an α-linkage[1,6,16,34].

3-O-β-D-Glucopyranosyl-L-rhamnopyranose. — Several lines of evidence favor a β-linkage between the monomeric units. The chemical shift of C-1′ (105 p.p.m.) would be abnormal for a carbon involved in an α-linkage (at neutral pD). Linkage of a rhamnopyranose ring to a glucopyranose ring results in a change in chemical shift for C-1′ of about +8 p.p.m., comparable to that obtained for C-1′ of a disaccharide with two glucopyranose rings[6]. The two resonances around 77 and 75 p.p.m. (C-5β, C-2β, and C-3β in D-glucose) are further evidence for a β-D-glucosyl unit in the disaccharide. Indeed, in this region, no resonance is present for L-rhamnose monomer, or can be expected to appear by glycosidic linkage of any carbon of this monomer to β-D-glucose. As such a linkage introduces a change in chemical shift of *ca.* +10 p.p.m. for the linked carbon of the reducing unit, a carbon of L-rhamnose linked to β-D-glucose should resonate above 80 p.p.m. Confirmation of the C-3 linkage of the L-rhamnose unit arises from the absence of a resonance at 74 p.p.m. and from the fact that two lines between 85 and 80 p.p.m. have chemical shift values separated by 2.5 p.p.m. In the L-rhamnose monomer, the C-3β and C-3α resonances are separated

TABLE I

ASSIGNMENT OF THE ^{13}C RESONANCES OF DISACCHARIDES OF THE D-GLUCOSYL-L-RHAMNOSE SERIES

	D-*Glucose*	L-*Rhamnose*	*3*-O-*β*-D-*Glucopyranosyl*-L-*rhamnopyranose*	*4*-O-*β*-D-*Glucopyranosyl*-L-*rhamnopyranose*	*2*-O-*β*-D-*Glucopyranosyl*-L-*rhamnopyranose*[c]
C-1′	—	—	105.0	104.4	105.3(α) 104.6(β)
C-1α	93.1	95.2	95.0	95.0	94.0
C-1β	97.0	94.7	94.6	94.6	93.9
C-2′	—	—	74.7	75.1	74.5
C-2α	72.5[a]	72.0	71.8	72.0	82.1
C-2β	75.2	72.5	72.3[b]	72.5	82.4
C-3′	—	—	76.9[a]	77.2[b]	77.0
C-3α	73.8	71.2	81.0	71.2	70.9
C-3β	77.0	74.0	83.5	74.0	74.3
C-4′	—	—	70.8	70.8	70.5
C-4α	70.7	73.4	72.5[b]	82.5	73.5
C-4β	70.7	73.2[a]	72.3[b]	82.0	73.2[b]
C-5′	—	—	76.9[a]	77.0[b]	76.7
C-5α	72.5[a]	69.5	69.5	68.0	69.3
C-5β	76.8	73.2[a]	73.0	71.6	73.8[b]
C-6′	—	—	61.9	61.9	61.7
C-6α	61.7	18.0[a]	18.1[a]	18.2[a]	17.9
C-6β	61.8	18.0[a]	18.1[a]	18.2[a]	17.9

[a]No differentiation observed in chemical shifts. [b]Uncertainty concerning relative assignments. [c]See also *footnote* p. 5.

by 2.8 p.p.m., whereas those of C-4β and C-4α and those of C-2β and C-2α are very similar (differences of 0.2 and 0.5 p.p.m., respectively). Moreover, no differential effect of linkage formation has been found for a linked carbon in the α- and β-forms of the reducing end of a disaccharide. The remaining resonances in the spectrum of this disaccharide can be easily interpreted in terms of those of the monomers, and the effects of the disaccharide formation[6]. The 74.7 p.p.m. value for C-2′, a difference of −0.5 p.p.m. with respect to the monomer, is due to the linkage of β-D-glucose in position 1. Similarly, C-4α and C-4β of L-rhamnose resonate at 72.5 and 72.3 p.p.m., respectively, undergoing an expected −0.9 p.p.m. change in chemical shift due[6] to the linkage at C-3. The C-5β, C-2β, and C-2α resonances can be identified as those at 73.0, 72.3, and 71.8 p.p.m., respectively, because formation of the β-(1→3)-linked disaccharide is expected to influence the chemical shifts of C-2 and C-5 less than that of C-4, as found for laminaribiose[1,6]. Finally, the relative intensities of the resonances between 73.0 and 70.8 p.p.m., which are all due to –CHOH groups, confirm the assignments discussed above and given in Table I. It is noteworthy that we did not observe a significant change in the chemical shift of C-2α of the L-rhamnose residue (HO-2 of L-rhamnose is axial) on disaccharide formation at position 3. Earlier studies of substituted D-galactose[20] or D-mannose[14] and a mannan[14] having alternate (1→3) and (1→4)-linked β-D-mannopyranose residues suggested a change in chemical shift

of *ca.* −4.5 p.p.m. for carbons with axial –OH groups in the β-position on formation of *O*-methyl or *O*-glucosyl linkages. For β-D-glucosyl-(1→3)-L-rhamnose, such an upfield shift would have resulted in two resonances at 68–68.5 p.p.m.

4-O-β-D-Glucopyranosyl-L-rhamnopyranose. — A β-(1→4)-linkage is clearly indicated by analysis of the ^{13}C-n.m.r. spectrum, especially by the resonances for C-1′ (104.4 p.p.m.), C-2′, C-3′, and C-5′ (75.1, 77.2, and 77.0 p.p.m., respectively) of the non-reducing D-glucose residue and by the two C-4 resonances (82.5 and 82.0 p.p.m. for the α and β anomer, respectively) of the reducing L-rhamnose residue. The 77.2 and 77.0 p.p.m. resonances cannot be unequivocally assigned to one of C-3′ or C-5′, just as in the case of C-3β and C-5β of D-glucose. Assignment of the resonances between 74.0 and 70.8 p.p.m. is somewhat more difficult. The C-5β resonance is assigned at 71.6 p.p.m., because it can be expected to undergo the same change in chemical shift (about −1.5 p.p.m.) due to the linkage as C-5β in the β-D-(1→4) disaccharide cellobiose[6]. The C-2β and C-2α resonances are assigned at 72.5 and 72.0 p.p.m., respectively, by assuming a negligible effect[6] on C-2 due to the linkage at C-4. As the linkage at C-4 is expected to influence the C-5 resonance more strongly than the C-3 resonance[6], the peaks at 74.0 and 71.2 p.p.m. resonances are assigned to C-3β and C-3α, respectively.

2-O-β-D-Glucopyranosyl-L-rhamnopyranose. — As in the glucose or mannose disaccharide series, this disaccharide has some peculiarities due to the location of the glycosidic linkage on the carbon neighbouring the C-1 anomeric position (Fig. 1). The population of the α anomer is about twice that of the β anomer in L-rhamnose, as previously demonstrated[21–23] by ^{1}H-n.m.r. data, and the C-1α and C-1β resonances are unequivocally assigned at 94.0 and 93.9 p.p.m., respectively, by comparison with the other disaccharides. These chemical shifts differ by about −1 p.p.m. with respect to the same resonances in the other disaccharides and L-rhamnose, due to the linkage at the neighbouring carbon, the hydroxyl group of which is axial. From the knowledge of the relative populations of the two anomers of L-rhamnose, it can be inferred that the 105.3 and 104.6 p.p.m. resonances are C-1′ resonances related to the α- and

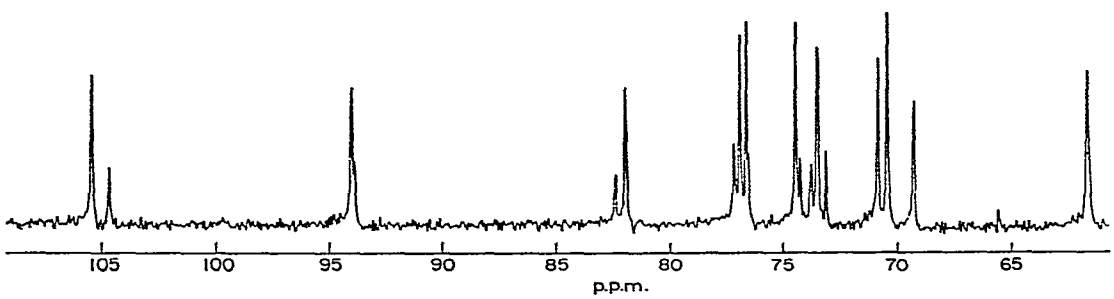

Fig. 1. ^{13}C-N.m.r. spectrum of 2-*O*-β-D-glucopyranosyl-L-rhamnopyranose: 45 mg/ml in D_2O, 32°, pD 7.0, spectral width 5 kHz, 8 K data points, cycle time 0.8 sec., pulse angle 45°, 54,668 transients. The methyl resonance of L-rhamnopyranose (17.9 p.p.m.) cannot be seen on this expanded spectrum.

β-anomeric forms of C-1, respectively. Similarly, the C-2β and C-2α resonances are located at 82.4 and 82.1 p.p.m., respectively. The intensity ratio I_α/I_β for the components of the C-1 and C-2 resonances is *ca.* 2.5 and is useful for assignment. Although the comparison is not quantitatively valid (due to short cycle times in averaging the free induction decays), the same ratio between the intensities of the so-called C-1′(α) and C-1′(β) resonances has a similar value. This splitting of the C-1′ carbon illustrates the high conformational sensitivity of the ^{13}C-n.m.r. technique: the passage from the equatorial to axial position of the OH linked to the anomeric carbon (C-1), a γ-substituent with respect to the C-1′ carbon, induces a +0.8 p.p.m. change in chemical shift of this latter carbon.

The evidence for the β-linkage is best given by the C-1′ (~105 p.p.m.), C-3′ (77.0 p.p.m.), and C-5′ (76.7 p.p.m.) resonances of the non-reducing D-glucose residue. The absence of any resonance between 73.0 and 71.0 p.p.m. provides further proof for a (1→2) glycosidic linkage, due to the well-known chemical shifts of C-2 in the L-rhamnose monomer (72.5 and 72.0 p.p.m. for C-2β and C-2α, respectively).

The three remaining resonances of the non-reducing unit (D-glucose) of the disaccharide are easily assigned by reference to the other disaccharides of this series: the resonances at 74.5, 70.5, and 61.7 p.p.m. are assigned to C-2′, C-4′, and C-6′, respectively. Complete assignment of the reducing unit (L-rhamnose) of the disaccharide is more difficult, except for the C-1, C-2, and C-6 resonances. The two resonances at 73.5 and 73.2 p.p.m. (intensity ratio, 2.4) are due to C-4α and C-4β, which are relatively insensitive to the linkage. The C-3α and C-5α resonances are clearly positioned at 70.9 and 69.3 p.p.m., respectively. The vicinal position of C-3α with respect to the linkage induces a small shift to high field (−0.3 p.p.m.) of the C-3α resonance, which is greater than that induced on C-5α (−0.2 p.p.m.), with respect to L-rhamnose monomer. Methylation in position 2 of α-D-mannose also produced different shifts to high field for C-3 (−0.3 p.p.m.) and C-5 (−0.1 p.p.m.)[14]. Methylation of the same position of β-D-mannose induced small shifts in the opposite direction for C-3 and C-5 (+0.4 and +0.3 p.p.m., respectively)[14]. Referring to the methylation of D-mannose, we tentatively assign the resonances at 74.3 and 73.8 p.p.m. to C-3β and C-5β, respectively*.

*A further resonance is present in this spectrum at 77.2 p.p.m. Its intensity, compared to that of all other resonances, corresponds to a β form. As it is very near the C-3′ resonance (77.0 p.p.m.) and has an intensity half that of the latter resonance, one could envisage a small conformational effect through four bonds (0.2 p.p.m.) according to the equatorial or axial position of the anomeric carbon of the L-rhamnose residue. In this case, the resonances at 77.2 and 77.0 p.p.m. should be the so-called C-3′(β) and C-3′(α) resonances, respectively. If true, a similar or larger effect should be observed for the C-2′ resonance positioned at 74.5 p.p.m. However, in this region, there are five resonances between 74.5 and 73.2 p.p.m., and one cannot determine relative intensities with confidence to confirm a possible conformational effect through three bonds. Only specific deuteration could give an unambiguous answer to this question and definitively confirm the assignment for this region.

A shoulder is also present on the high-field side of the C-5′ resonance (76.7 p.p.m.) and is separated from it by 0.1 p.p.m. By an argument similar to that developed for the two resonances at 77.2 and 77.0 p.p.m., one could tentatively assign the resonances at 76.7 and 76.6 p.p.m. to the so-called C-5′(α) and C-5′(β) resonances, respectively.

Galactose–rhamnose series

The ^{13}C-n.m.r. spectra of D-galactose have been reported by Voelter *et al.*[20,31,35] and also by Gorin[14]. Our assignments in Table II differ in some respects from those of Gorin: the most-important differences are in the chemical shift of C-2α and a reversal of the relative assignments of C-3α and C-4β. Besides the consideration of the relative populations of the two anomers, we shall present further evidence favoring our assignment. Haverkamp *et al.*[36,37] also studied, by ^{13}C- and ^{1}H-n.m.r., permethylated galactopyranoses and permethylated disaccharides composed of D-glucose, or D-galactose, or both residues. In Table II, the three disaccharides of this series are completely assigned, and the chemical shifts of L-rhamnose are also given for comparison.

TABLE II

ASSIGNMENT OF THE ^{13}C RESONANCES OF DISACCHARIDES OF THE D-GALACTOSYL-L-RHAMNOSE SERIES

	D-*Galactose*	L-*Rhamnose*	*4*-O-*β*-D-*Galactopyranosyl*-L-*rhamnopyranose*	*3*-O-*β*-D-*Galactopyranosyl*-L-*rhamnopyranose*	*2*-O-*β*-D-*Galactopyranosyl*-L-*rhamnopyranose*[c]
C-1′	—	—	104.9	105.5	105.9(α) 105.1(β)
C-1α	93.4	95.2	95.0	95.0	94.1
C-1β	97.7	94.7	94.6	94.5	93.9
C-2′	—	—	72.9	72.4[b]	72.2
C-2α	70.3[a]	72.0	72.0	71.9	81.7
C-2β	73.0	72.5	72.5	72.4[b]	82.4
C-3′	—	—	74.0[b]	73.8	73.7
C-3α	69.4	71.2	71.2	81.0	71.1
C-3β	73.9	74.0	74.0[b]	83.4	74.2
C-4′	—	—	69.8	69.9	69.7
C-4α	70.4[a]	73.4	82.3	72.4[b]	73.6[b]
C-4β	69.8	73.2[b]	81.9	72.4[b]	73.3
C-5′	—	—	76.4	76.3	76.2
C-5α	71.6	69.5	68.1	69.5	69.3
C-5β	76.2	73.2[b]	71.8	73.0	73.6[b]
C-6′	—	—	62.1	62.3	62.2
C-6α	62.3	18.0[b]	18.2[b]	18.1[b]	18.1
C-6β	62.1	18.0[b]	18.2[b]	18.1[b]	17.9

[a]Assignments which may be reversed. [b]No difference observed in chemical shifts. [c]See also footnote p. 8.

Evidence for a β-linkage of C-1′ of D-galactose in each disaccharide of this series clearly appears from the C-1′ resonance at *ca.* 105 p.p.m., as well as from the other resonances of the non-reducing unit of those disaccharides. The only disaccharide studied to date where the galactose moiety is α-linked is melibiose (6-*O*-α-D-galactopyranosyl-D-glucopyranose). Its C-1′ resonance is found[35,38] at 99.4 p.p.m., whereas in lactose (4-*O*-β-D-galactopyranosyl-D-glucopyranose) the same resonance appears[35] at 104.1 p.p.m. Finally, in each disaccharide, the presence of a resonance

around 69.8 p.p.m., belonging to the non-reducing unit (in fact, C-4'), favors the relative assignment of the C-4β and C-3α resonances of the D-galactose monomer at 69.8 and 69.4 p.p.m., respectively. Only the resonances of the reducing unit remain for assignment in the individual disaccharides.

4-O-β-D-Galactopyranosyl-L-rhamnopyranose. — The absence of any resonance between 74.0 and 72.9 p.p.m., as well as the appearance of two resonances at 82.3 (C-4α) and 81.9 p.p.m. (C-4β), confirm the chemical evidence for a glycosidic linkage involving C-4 of L-rhamnose. The direct comparison with this latter monomer enables the assignment of nearly all the resonances of the reducing end of the disaccharide. The C-1, C-2, C-3, and C-6 resonances are very close to their values in the monomer. The C-3β resonance cannot be distinguished from that of C-3', while the C-5 chemical shift is much more affected than the others by the linkage at C-4 (−1.4 p.p.m. for C-5α and C-5β). The asymmetric influence of linkage at C-4 of L-rhamnose was also found in the D-glucopyranosyl-L-rhamnopyranose series (*vide supra*) and can be compared with the *O*-methylation on C-4 of D-mannose[14] and D-glucitol[30].

3-O-β-D-Galactopyranosyl-L-rhamnopyranose. — The best evidence for the involvement of C-3 of L-rhamnose in the glycosidic linkage is given by the presence of two resonances between 84 and 81 p.p.m., and especially by the difference in their chemical shifts (2.4 p.p.m.). As seen in the L-rhamnose monomer, only the two C-3 resonances have a comparable difference in chemical shift (2.8 p.p.m.). The same argument was also used earlier (*vide supra*) to identify 3-*O*-β-D-glucopyranosyl-L-rhamnopyranose. The involvement of this C-3 in the glycosidic linkage is also corroborated by the absence of any resonance around the normal position of the C-3α carbon of L-rhamnose (71.2 p.p.m.).

The C-1 and C-6 resonances of the reducing unit are as usual clearly defined. However, the remaining resonances will be discussed taking into account their relative intensities and the expected effect due to glycoside formation at C-3. The resonances at 73.0 and 69.5 p.p.m. correspond very well to C-5β and C-5α of L-rhamnose, which are not noticeably influenced by the linkage at C-3. Similarly, C-2α can be positioned at 71.9 p.p.m., corresponding to the position in the monomer (72.0 p.p.m.). Finally, the 72.4 p.p.m. resonance is too intense to be due to a single carbon, and is much broader than the others in the spectrum. Therefore, it must be a composite resonance due to C-2β, C-4α, C-4β, and C-2', taking into account the very small effect on C-2 (−0.1 p.p.m.) and the expected −1.0 p.pm. change in chemical shift on C-4 due to the linkage on C-3, and the expected effect of glycosidic linkage on C-2' (−0.6 p.p.m.). The intensity of this resonance and a shoulder on its high-field side very strongly justify its composite assignment.

2-O-β-D-Galactopyranosyl-L-rhamnopyranose. — The splitting of the C-1' resonance, as well as the presence of two resonances above 80 p.p.m. (82.4 and 81.7 p.p.m. for C-2β and C-2α, respectively) separated by 0.7 p.p.m., support the involvement of C-2 of L-rhamnose in the glycosidic linkage of this disaccharide. According to their relative intensities, the resonances at 105.9 and 94.1 p.p.m. are

due to C-1′(α) and C-1α, whereas 105.1 and 93.9 p.p.m. refer to C-1′(β) and C-1β. No problem exists for the assignment of C-4′, C-5′, C-6′, and the two C-6 resonances. As expected, C-2′ undergoes a −0.8 p.p.m. change in chemical shift. The (1→2)-linkage induces a larger change in chemical shift of C-2′ than any other linkage (*vide supra*, 2-*O*-β-D-glucopyranosyl-L-rhamnopyranose, −0.7 p.p.m.). A small differential influence of the C-2 linkage on C-3α and C-3β enables us to assign the 71.1 and 74.2 p.p.m. resonances to C-3α and C-3β, respectively. The intensity ratio of these two lines is in agreement with their relative assignments. Thus, in this disaccharide, the linkage at C-2 induces a −0.2 and a +0.2 p.p.m. change in chemical shift for C-3α and C-3β, respectively. The three resonances between 73.7 and 73.3 p.p.m. must be discussed in greater detail. All the remaining carbons to assign (C-3′, C-4α, C-4β, and C-5β) can be expected to resonate in this limited region, by comparison with other disaccharides of this series and with the monomers, as well as in view of the expected negligible effect of the C-2 linkage. According to the relative intensities of the resonances and their chemical shifts relative to those of the monomers, that at 73.7 p.p.m. can be logically assigned to C-3′, that at 73.3 p.p.m. must belong to a β form (C-4β or C-5β), and that at 73.6 p.p.m. has to be composite (C-4α and C-4β or C-5β). Tentatively, we assign C-5β with C-4α at 73.6 p.p.m., because C-5 is closer to the anomeric center than C-4 and can therefore be more sensitive to a change from an equatorial to an axial hydroxyl group*.

Acetamido-containing disaccharides

The ^{13}C-n.m.r. spectra of monomeric units present in these disaccharides, *i.e.*, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, and L-rhamnose, have been already reported by several authors[18,27,31,33]. The assignments are listed in Table III for comparison with those of the disaccharides.

2-Acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucopyranose. — The resonances at 57.4 and 55.0 p.p.m. correspond very well to C-2β and C-2α of 2-acetamido-2-deoxy-D-glucose as reducing residue, whereas that at 56.9 p.p.m. has to be due to C-2′ of the non-reducing unit. In fact, only resonances due to C-2 are present in this region and the value of 56.9 p.p.m. for C-2′ demonstrates a β linkage between the two glucosamine residues, as it is well known that β-glycosylation induces a high-field shift for C-2′ (−1.1 p.p.m. in this case). To justify an α-glycoside, a change of +1.6 p.p.m. should be put forward, as C-2α of the monomer resonates at 55.3 p.p.m. The presence of two resonances around 77 and 75 p.p.m., as well as the position of the C-1′ resonance (102.7 p.p.m. at neutral pD), provide

*As in 2-*O*-β-D-glucopyranosyl-L-rhamnopyranose, a further resonance is present at 76.8 p.p.m., the intensity of which corresponds to a β form. But it cannot be assigned by the type of argument used for the former disaccharide. Indeed, its difference in chemical shift with respect to the nearest resonance is too important (+0.6 p.p.m.) to be justified by a conformational effect through three or four bonds. On the other hand, another resonance is also present on the high-field side of the C-2′ resonance (72.2 p.p.m.), separated from this latter by only 0.1 p.p.m. Following the relative intensities of these two resonances and their separation (0.1 p.p.m.), the resonances at 72.2 and 72.1 p.p.m. should be due to the so-called C-2′(α) and C-2′(β), respectively.

TABLE III

ASSIGNMENT OF THE ^{13}C RESONANCES OF DISACCHARIDES RELATED TO 2-ACETAMIDO-2-DEOXY-D-GLUCOSE, 2-ACETAMIDO-2-DEOXY-D-GALACTOSE, AND L-RHAMNOSE

	2-Acetamido-2-deoxy-D-glucose[a]	*2-Acetamido-2-deoxy-D-galactose*[a]	*L-Rhamnose*	*2-Acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucopyranose*	*2-Acetamido-6-O-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-2-deoxy-D-galactopyranose*	*3-O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-L-rhamnopyranose*
C-1′	—	—	—	102.7	103.3	103.9
C-1α	92.1	92.2	95.2	91.8	92.2	94.8
C-1β	96.2	96.5	94.7	96.2	96.6	94.5
C-2′	—	—	—	56.9	53.6	57.0
C-2α	55.3	51.4	72.0	55.0	51.5	71.8
C-2β	58.0	54.9	72.5	57.4	55.0	72.3[b]
C-3′	—	—	—	74.7	72.2	74.9
C-3α	72.0	68.6	71.2	70.6	68.5	81.0
C-3β	75.2	72.3	74.0	73.8	72.2	83.4
C-4′	—	—	—	71.0	69.0[b]	71.0
C-4α	71.4	69.7	73.4	81.1	69.7	72.3[b]
C-4β	71.2	69.0	73.2[b]	80.7	69.0[b]	72.3[b]
C-5′	—	—	—	77.2	76.3	76.9
C-5α	72.8	71.6	69.5	71.6	70.3	69.8
C-5β	77.2	76.3	73.2[b]	75.8	75.1	73.3
C-6′	—	—	—	61.8	62.2	61.8
C-6α	61.9	62.4	18.0[b]	61.3[b]	69.0[b]	18.0[b]
C-6β	62.0	62.2	18.0[b]	61.3[b]	69.0[b]	18.0[b]
(C=O)′	—	—	—	175.8[b]	175.9[b]	176.1
(C=O)α	175.7	176.1	—	175.7	176.1	—
(C=O)β	175.9	175.8	—	175.8[b]	175.9[b]	—
$(CH_3)'$	—	—	—	23.4[b]	23.5[b]	23.5
$(CH_3)\alpha$	23.3	23.3	—	23.2	23.2	—
$(CH_3)\beta$	23.5	23.4	—	23.4[b]	23.5[b]	—

[a]From Ref. 33. [b] No differentiation in chemical shift could be observed.

further evidence for the β-glycosidic link. Carbonyl and methyl resonances of the acetamido group are assigned from knowledge of the β-linkage and from the relative peak intensities: 175.8 (C=O) and 23.4 p.p.m. (CH_3) for the β form of the reducing unit and for the non-reducing unit, and 175.7 (C=O) and 23.2 p.p.m. (CH_3) for the α form of the reducing unit.

Additional evidence for the β-(1→4)-linkage comes from the C-1′ resonance (102.7 p.p.m.), which, however, comes at higher field than in the corresponding D-glucose derivative, due to the influence of the acetamido group (Table I). Attachment at C-4 is confirmed by the appearance of two resonances very close to one another (0.4 p.p.m.) around 81 p.p.m., and the absence of two resonances around 71.3 p.p.m. The remaining resonances of the non-reducing unit are now easily assigned: C-3′, C-4′, C-5′, and C-6′ at 74.7, 71.0, 77.2, and 61.8 p.p.m., respectively. Only a small effect (−0.5 p.p.m.) is manifest on C-3′ due to the linkage at C-1′. As linkage at C-4 produces a change in chemical shift of *ca.* −1.5 p.p.m. for the neighbouring carbons (as in cellobiose[1,16,38]), the resonances at 73.8 and 70.6 p.p.m. are assigned to C-3β and C-3α, respectively, and those at 75.8 and 71.6 p.p.m. to C-5β and C-5α, respectively. Finally, in this spectrum, a difference in chemical shift of −0.5 p.p.m. is observed for C-6 and C-6′. This seems to be due to the β-(1→4)-linkage rather than to the presence of the acetamido groups, as we[38] and other authors[1,16] observed the same phenomenon for C-6 resonances of cellobiose. The upfield shift of these latter resonances was assigned by Dorman and Roberts[16] to an intramolecular steric perturbation.

2-Acetamido-6-O-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-2-deoxy-D-galactopyranose. — For reasons similar to those given for the previous acetamido-bearing disaccharide, we infer a β-linkage between the two monomeric units. The position of the C-1′ resonance at 103.3 p.p.m. and the presence of the C-5′ resonance at 76.3 p.p.m. (corresponding with that of C-5β of 2-acetamido-2-deoxy-D-galactose) provide the best evidences for a β linkage. Two arguments favor the involvement of the C-6 of the reducing unit in the glycosidic linkage: the high intensity of the resonance line at 69.0 p.p.m., and the close similarity of the chemical shifts of all carbons of the reducing unit with respect to the monomer unit (maximal difference: ±0.1 p.p.m.), with the exception of C-5 and C-6. C-5α and C-5β undergo a change in chemical shift of *ca.* −1.3 p.p.m., as expected for a carbon in a β-position with respect to a linkage. According to these observations and the relative intensities of the peaks, the complete assignment of this disaccharide is easily made, as presented in Table III. Notice the change in chemical shift of C-6 by +7.0 p.p.m. due to linkage formation, and that of −1.3 p.p.m. for C-2′ (with respect to the C-2β carbon in the monomer) due to the involvement of C-1′ in the β-(1→6)-linkage.

3-O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-L-rhamnopyranose. — The same lines of evidence used for 3-*O*-β-D-glucopyranosyl-L-rhamnopyranose favor both a β linkage and the involvement of C-3 of the reducing unit: C-1′ at 103.9 p.p.m., and two resonances separated by 2.4 p.p.m. in the range 80–85 p.p.m., two resonances of primed carbons around 77 and 75 p.p.m. (76.9 and 74.9 p.p.m. for C-5′ and C-3′,

respectively, in good correspondence with C-5β at 77.2 p.p.m. and C-3β at 75.2 p.p.m. for 2-acetamido-2-deoxy-D-glucose). The main differences between the spectrum of this disaccharide and that of 3-*O*-β-D-glucopyranosyl-L-rhamnopyranose arises from the presence of an acetamido group at C-2′ of the non-reducing unit. The C-2′ chemical shift (57.0 p.p.m.) results from the combined effects of the acetamido group at C-2′ and the involvement of C-1′ in a β linkage. The β linkage is further confirmed by the chemical shift of C-2′ (57.0 p.p.m.), a −1.0 p.p.m. change due to glycoside formation. For an α linkage, this resonance would be expected between 54 and 55 p.p.m. (*vide supra*). The −1.1-p.p.m. change in chemical shift of C-1′ with respect to that of 3-*O*-β-D-glucopyranosyl-L-rhamnopyranose is essentially due to a β-effect on C-1′ by *N*-acetylation of C-2′ (by comparison of D-glucose with 2-acetamido-2-deoxy-D-glucose). No assignment problem remains for the resonances of the non-reducing unit, including those of carbonyl and methyl carbons. The region between 73.3 and 71.0 p.p.m. must be considered more carefully (the 69.8-p.p.m. line is unambiguously due to the C-5α resonance of the L-rhamnose residue). The assignment which follows is given taking into account the spectrum of the related 3-*O*-β-D-glucopyranosyl-L-rhamnopyranose and the relative populations of the α and β forms of L-rhamnose. On this basis, the 73.3- and 71.8-p.p.m. resonances are due to β and α forms, respectively. For this reason, they are assigned to C-5β and C-2α of the L-rhamnose residue. Moreover, the ratio of the peak intensities at 69.8 and 73.3 p.p.m. agrees very well with that expected from the relative populations of the α and β forms of L-rhamnose. On the contrary, the resonance at 72.3 p.p.m. must be composite due to its high intensity. As C-3 of the L-rhamnose residue is involved in the glycosidic linkage, C-2β, C-4α, and C-4β resonances are all assigned at 72.3 p.p.m.

CONCLUSION

The analysis of the ^{13}C-n.m.r. spectra of the D-glucopyranosyl-L-rhamnopyranose and D-galactopyranosyl-L-rhamnopyranose series, and some related acetamido-disaccharides, leads to conclusions about the changes in chemical shift induced by disaccharide formation and the influence of an acetamido group in one or both of the constitutive units. A comparison is also possible between the disaccharides studied here and glucose disaccharides.

When β-D-glucose is involved in a disaccharide as non-reducing unit, only its C-1′ and C-2′ resonances are changed by more than 0.3 p.p.m. with respect to the monomer alone. This is valid for either D-glucose or L-rhamnose as reducing unit. However, the magnitudes of the changes at C-1′ and C-2′ are strongly affected by the nature of the reducing unit. Indeed, whereas, in the glucose disaccharides, the Δ-value* of C-2′ becomes increasingly lower as the linkage between the two D-glucose residues goes from β-(1→6) to β-(1→4), β-(1→3), and β-(1→2) (−1.0, −0.9, −0.6, and

*Δ-value of a given carbon is defined as the difference between its chemical shift in a disaccharide with respect to that of the monosaccharide.

−0.3 p.p.m., respectively), the reverse is observed for the three disaccharides of the glucose–rhamnose series. Moreover, replacement of D-glucose by L-rhamnose as reducing unit increases the downfield shift on C-1′ by *ca.* 1 p.p.m. in disaccharides having β-(1→3) and β-(1→4) linkages.

No important influence of the nature of the non-reducing unit (D-glucose, D-galactose, and 2-acetamido-2-deoxy-D-glucose) can be seen on the chemical shifts of the carbons of the reducing unit (L-rhamnose, in this case). Glycoside formation at position 3 or 4 of the L-rhamnose reducing unit has a differential influence on its neighbouring carbons. The higher-number carbon (4 or 5) is always more affected than the lower-number carbon (2 or 3). It is the reverse when the substitution is made on position 2, probably because the anomeric centre is neighbouring to the substituted carbon. Moreover, in the latter disaccharide, a differential effect is experienced by the C-3α and C-3β carbons, which respectively undergo a small, high-field (−0.3 p.p.m.) and down-field shift (+0.3 p.p.m.) due to the linkage at C-2. Such a differential effect was previously observed with methylation of D-mannose and D-glucitol[30]. Finally, in both the galactosyl–rhamnose and glucosyl–rhamnose series, the increase of chemical shift of the substituted carbon of the reducing unit is nearly the same for 2- and 3-*O*-β-substitution (+9.5 to 9.9 p.p.m.), but a little less-pronounced (+8.7 to 9.1 p.p.m.) for 4-*O*-β-substitution. The presence of an acetamido group in position 2 of the non-reducing unit has no particular influence on the substituted C-3 carbon of L-rhamnose. The β-(1→4) linkage of two acetamido-containing glucose residues in place of two glucose residues increases by +0.7 p p.m. the change in chemical shift undergone by the C-4 resonance of the reducing unit.

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