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DETERMINATION OF THE LINKAGES OF DISACCHARIDES CONTAINING A 2-ACETAMIDO-2-DEOXY SUGAR UNIT BY SOLVENT EFFECTS IN CIRCULAR DICHROISM*

HELEN R. DICKINSON, PHILLIP L. CODUTI†, AND C. ALLEN BUSH
Department of Chemistry, Illinois Institute of Technology, Chicago, Illinois 60616 (U. S. A.)
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ABSTRACT

The circular dichroism spectra of 2-acetamido-2-deoxy sugars in 1,1,1,3,3,3-hexafluoro-2-propanol (F_6Pr-2-ol) solutions show a positive band in the n–π^* region (209 nm) in contrast to a negative c.d. band in water solution. This difference is interpreted as an indication of a change in the average orientation of the hydroxyl groups adjacent to the amide group. C.d. spectra of 2-acetamido-2-deoxy sugars having a methyl group at O-1 and O-3 confirm this interpretation and suggest that the c.d. spectrum of a disaccharide in F_6Pr-2-ol reflects strongly the disaccharide linkage. Large differences in the c.d. spectra of (1→4) and (1→6)-linked disaccharides in this solvent lead to rules for distinguishing the linkages of the disaccharides.

INTRODUCTION

We have recently undertaken studies of the c.d. spectra of oligosaccharides containing 2-acetamido-2-deoxy sugars in order to establish which structural and conformational features of these molecules are reflected in the spectra. The c.d. spectra determined on water solutions of 2-acetamido-2-deoxy-D-glucose and -D-galactose show[1] similar, negative c.d. bands due to the amide group n–π^* transition at 209 nm. This c.d. band is rather insensitive to changes in the anomeric configuration of either the free sugars or their methyl glycosides. Furthermore, the band is only slightly modified in spectra of disaccharides having a (1→4) or (1→6) linkage. A band due to the π–π^* transition of the amide group at 190 nm is enhanced in (1→4)-linked disaccharides; the 190-nm band[1,2] is small or negative in (1→3)-linked polysaccharides and in 2-acetamido-2-deoxy sugars having a methyl substituent at O-3.

Theoretical calculations of the rotational strength of the n–π^* band in spectra of 2-acetamido-2-deoxy sugars indicate that the asymmetric electrostatic field responsible for the n–π^* optical activity arises mainly from the influence of the

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†Present address: Inland Steel Research Laboratories, East Chicago, Indiana 46312, U. S. A.

hydroxyl groups adjacent to the amide chromophore[3]. The dominant perturbation arises from the hydroxyl group at C-3 with a lesser contribution from the hydroxyl group at C-1. The details of the electrostatic perturbation depend on the orientations of these hydroxyl groups, which are specified by the dihedral angles about the respective C–O bonds[3]. Therefore, we have proposed that a change from water to solvents having different, hydrogen-bonding properties might be expected to cause substantial changes in the c.d. spectra of 2-acetamido-2-deoxy sugars. The data of Fig. 2 of the publication of Yeh and Bush[3] indicate that changes in dihedral angles about the C-3–O bond could lead to changes in the sign of the c.d. spectrum at 209 nm. In the present communication, we report the c.d. spectra of several derivatives and disaccharides of 2-acetamido-2-deoxy sugars in solution in 1,1,1,3,3,3-hexafluoro-2-propanol (F_6Pr-2-ol), a solvent that is primarily a hydrogen-bond donor.

RESULTS

In Table I, the extinction coefficients and wavelength of absorbance maxima for 2-acetamido-2-deoxy-D-glucose (**1**) and -D-galactose (**2**) in water and in F_6Pr-2-ol are reported. The modest differences between the absorption spectra of these two compounds in both solvents are only slightly outside the experimental error, and are probably not significant.

TABLE I

U.V. ABSORBANCE PARAMETERS FOR 2-ACETAMIDO-2-DEOXY-D-GLUCOSE (**1**) AND 2-ACETAMIDO-2-DEOXY-D-GALACTOSE (**2**) IN WATER AND 1,1,1,3,3,3-HEXAFLUORO-2-PROPANOL

Compound	*Solvent*	λ_{max} *(nm)*	*Extinction coefficient*
1	H_2O	189.0	9000
	F_6Pr-2-ol	187.5	9400
2	H_2O	189.5	9500
	F_6Pr-2-ol	188.0	8800

The c.d. bands of both **1** and **2** show a reversal in sign (Figs. 1 and 2), from a negative n–π^* band in water to a positive band in F_6Pr-2-ol. The wavelength position and absolute magnitude of the c.d. bands are similar for these two solvents.

The c.d. spectra of methyl 2-acetamido-2-deoxy-α- (**3**) and -β-D-glucopyranoside (**4**) (Fig. 3) and methyl 2-acetamido-2-deoxy-α- (**5**) and -β-D-galactopyranoside (**6**) in F_6Pr-2-ol show, for the α anomers, a c.d. band at 209 nm that is positive, as it is for the free sugars. For the β anomers, the c.d. spectrum is intermediate between those of the free sugar in water and in F_6Pr-2-ol, **6** showing a small, positive band at 209 nm, and **4** showing essentially no c.d. at that wavelength.

The c.d. spectra of two disaccharides, namely 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucose (di-*N*-acetylchitobiose) (**7**) and 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucose (**8**)

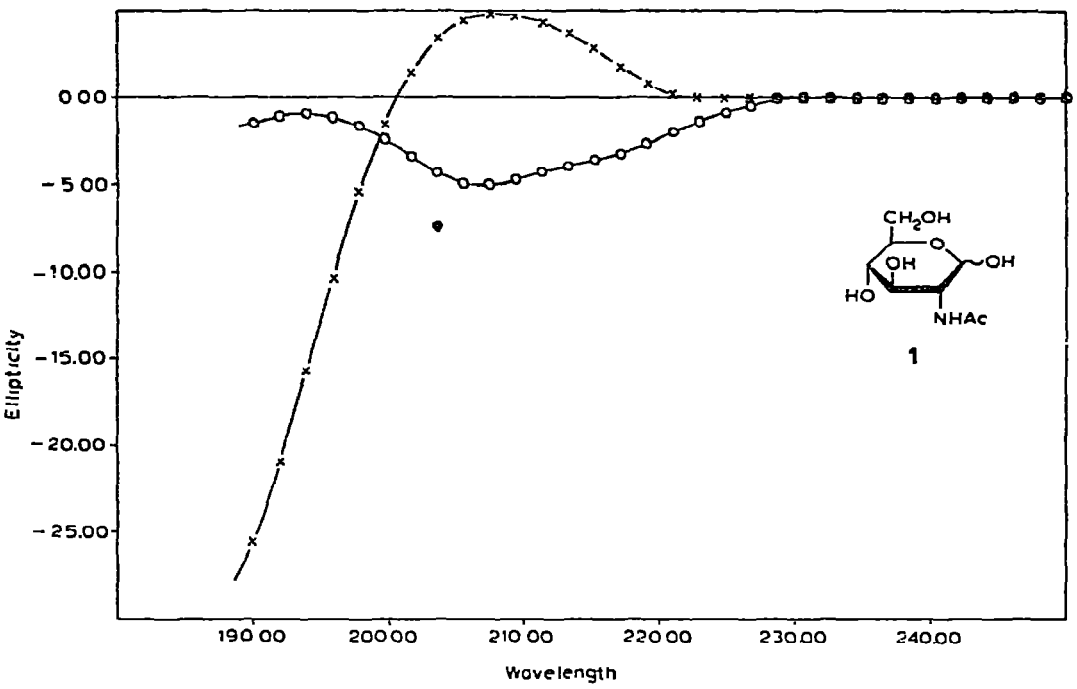

Fig. 1. C.d. (in units of molar ellipticity $\times 10^{-3}$) of 2-acetamido-2-deoxy-D-glucose (1) in H_2O (-O-O-) and in F_6Pr-2-ol (-●-●-).

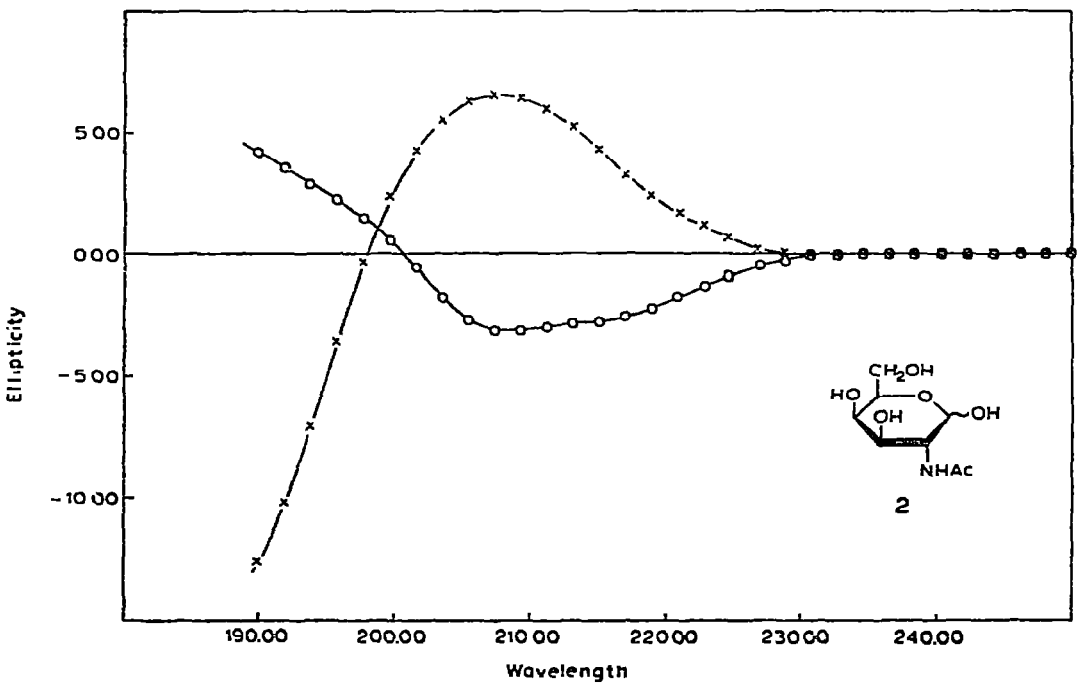

Fig. 2. C.d. (in units of molar ellipticity $\times 10^{-3}$) of 2-acetamido-2-deoxy-D-galactose (2) in H_2O (-O-O-) and in F_6Pr-2-ol (-●-●-).

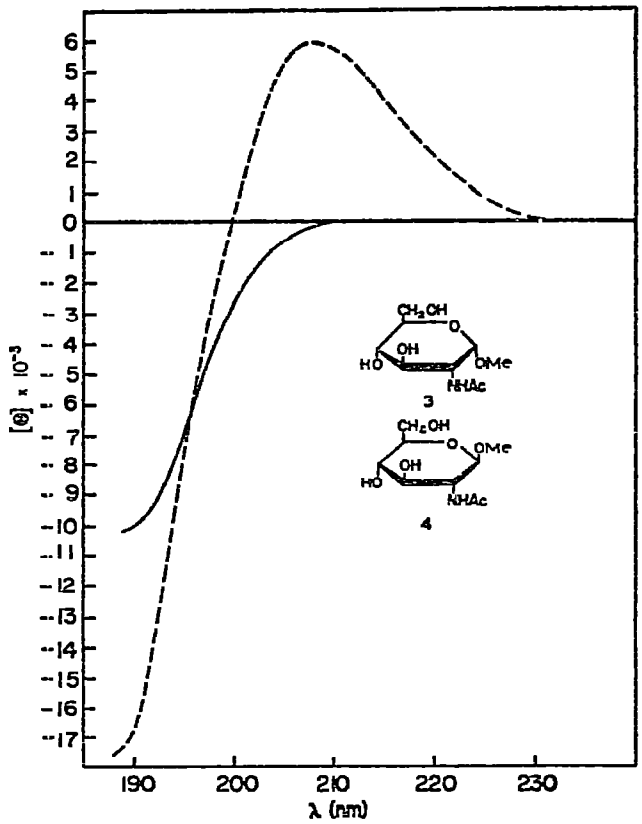

Fig. 3. C.d. of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (3) (– – –) and methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (4) (———) in F_6Pr-2-ol.

composed only of 2-acetamido-2-deoxy-D-glucose units, in F_6Pr-2-ol, are shown in Fig. 5. For the β-D-(1→6)-linked compound **6** in F_6Pr-2-ol, the c.d. spectrum shows a positive band at 209 nm; the magnitude of this band, expressed on a per residue basis, is intermediate between that of the free sugar (Fig. 1) and that of **4** (Fig. 3), both in F_6Pr-2-ol. For the β-D-(1→4)-linked isomer (**5**) in F_6Pr-2-ol, the c.d. spectrum is quite different from that of the component residues in the same solvent. In water, the c.d. spectra of the two disaccharides **5** and **6** are similar to each other and to that[1] of *4*.

The hypothesis that led us to perform these experiments suggests the importance of substituents at O-3. The data reported in Fig. 6 show that methyl 2-acetamido-2-deoxy-3-*O*-methyl-β-D-glucopyranoside (**9**) in F_6Pr-2-ol has a c.d. spectrum quite similar to that of the water solution. The solvent effect is absent when both hydroxyl groups adjacent to the amide group have methyl substituents.

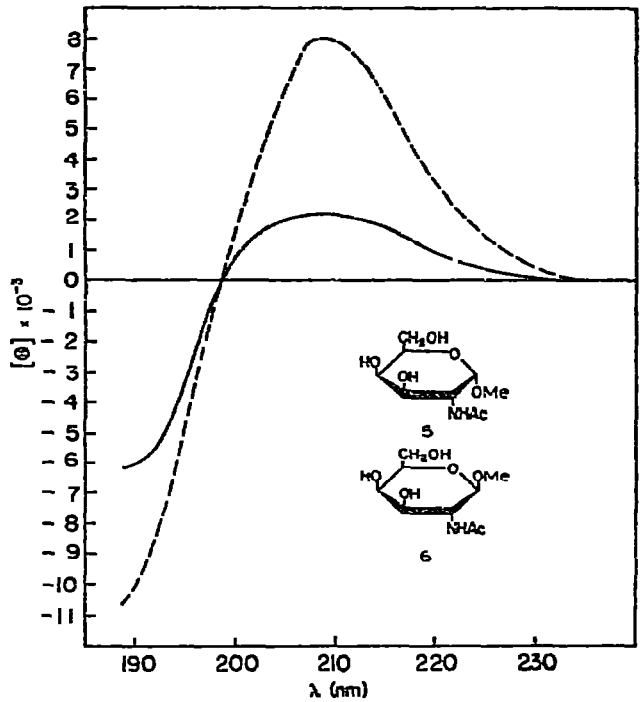

Fig. 4. C.d. of methyl 2-acetamido-2-deoxy-α-D-galactopyranoside (5) (— — —) and methyl 2-acetamido-2-deoxy-β-D-galactpyranoside (6) (———) in F_6Pr-2-ol.

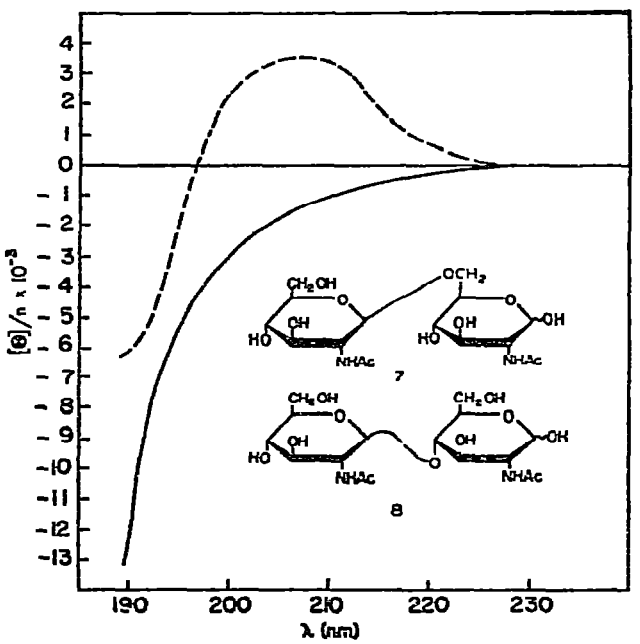

Fig. 5. C.d. of 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucose (di-*N*-acetyl-chitobiose) (7) (———) and of 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucose (8) (— — —) in F_6Pr-2-ol. Units are molar ellipticity per residue.

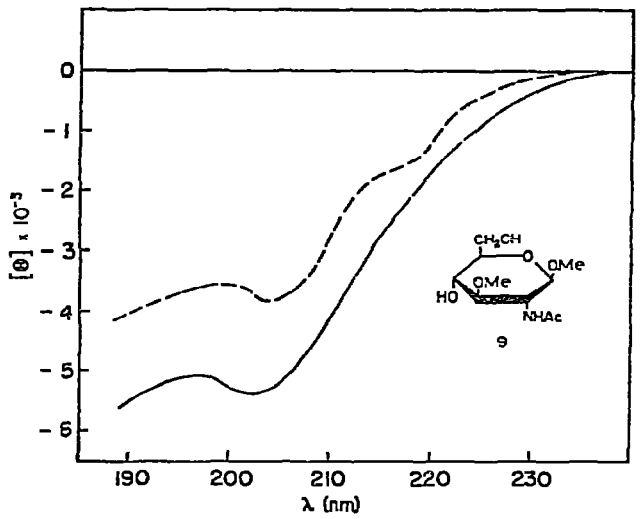

Fig. 6. C.d. of methyl 2-acetamido-2-deoxy-3-*O*-methyl-β-D-glucopyranoside (**9**) in water (———) and in F_6Pr-2-ol (— — —).

DISCUSSION

Sign changes of the n–π band of* **1** *and* **2** *in water and F_6Pr-2-ol solutions.* — A number of possible explanations of these changes may be eliminated: (a) The sign change cannot result solely from a change in the anomeric equilibria of the sugars, since the c.d. band at 209 nm is negative for both anomers of **1** and **2** in water. Likewise, in water, both methyl α- and β-D-glycosides of **1** and **2** (**3**, **4**, **5**, and **6**) have a negative c.d. in the n–π* region[1]. Finally, the methyl α-D-glycosides **3** and **5** show a sign change between water and F_6Pr-2-ol solutions (Figs. 3 and 4). (b) A major change in the preferred conformation of the pyranose ring between water and F_6Pr-2-ol may also be eliminated, because measurements of vicinal, n.m.r. coupling-constants on solutions of **1** in dimethyl sulfoxide and in F_6Pr-2-ol show that the preferred ring conformation is the $^4C_1$ chair conformation, which is also the conformation found for a water solution as well as the crystalline state[5]. (c) A third possible cause that may be eliminated is a major change in the orientation of the chromophoric amide group between water and F_6Pr-2-ol solutions resulting from a rotation about the C–N bond. The amide proton has a *trans* relationship to H-2, as shown by the large H-C–N-H coupling constant in water, dimethyl sulfoxide, and F_6Pr-2-ol solutions[4]. A similar orientation for the amide group is also found[5] in crystalline **1**. (d) Finally, the possibility that there is a substantial change in the electronic structure of the amide group chromophore resulting from differing solvation by water and by F_6Pr-2-ol may also be excluded. The carbonyl oxygen atom of the amide group is a hydrogen-bond acceptor. Therefore, the chromophore is expected to interact similarly with water and F_6Pr-2-ol. This interpretation is supported by the

data reported in Table I, which show that the extinction coefficients and absorption maxima of the π–π^* transition (near 190 nm) are quite similar for water and F_6Pr-2-ol solutions. Likewise, the c.d. bands due to the n–π^* transition occur at similar wavelengths for water and for F_6Pr-2-ol solutions (see Figs. 1 and 2).

Consequently, the most adequate explanation for the change in sign of the n–π^* c.d. band between water and F_6Pr-2-ol solutions is based on a difference in the average orientation of the hydroxyl groups adjacent to the amide group. Furthermore, it is suggested that the hydroxyl group that has the greatest influence is that at C-3. This proposal is supported by the theoretical calculations of Yeh and Bush[3] and by the observation that the change of sign between water and F_6Pr-2-ol solutions is absent for **7** (Fig. 6). The two methoxyl groups vicinal to the amide group are hydrogen-bond acceptors only. Therefore, the hydrogen-bond accepting power of the solvent becomes irrelevant, and the c.d. spectra are similar for water and F_6Pr-2-ol solutions. The dominant influence of OH-3 over OH-1 is demonstrated by the c.d. data of **3**, **4**, **5**, and **6** (Figs. 3 and 4). In these compounds, OH-3 is free to reflect the differing perturbations of water and F_6Pr-2-ol. Both α-D-glycosides **3** and **5** show this sign change. That the effect cannot be entirely attributed to OH-3 is shown by the c.d. curves of the β-D-glycosides **4** and **5**, which show the solvent effect to a lesser extent.

The c.d. spectrum of disaccharides in F_6Pr-2-ol solution. — The c.d. spectrum of the β-D-(1→6)-linked disaccharide (**8**) in F_6Pr-2-ol solution is essentially the average of the spectra of free **1** and of the methyl β-D-glycoside **6** in F_6Pr-2-ol (compare Figs. 1, 3, and 5). This implies that the two residues of **8** contribute independently to the c.d. of the F_6Pr-2-ol solution. A similar interpretation has been given for the c.d. spectra of water solutions of the β-D-(1→6)-linked (**7**) and β-D-(1→4)-linked (**8**) disaccharides[1]. In contrast, di-*N*-acetylchitobiose (**7**) in F_6Pr-2-ol solution shows a weak, negative c.d. value at 209 nm, with no clear c.d. trough at that wavelength. Our interpretation of these data is that the amide residue at the nonreducing, terminal sugar residues contributes a c.d. characteristic of **4**, which is essentially zero (see Fig. 3), and that the reducing, terminal sugar residue is contributing a negative c.d. value. A possible explanation for the latter contribution could be an intramolecular hydrogen-bond between OH-3 of this residue and the pyranose-ring, oxygen atom of the nonreducing residue. Such a hydrogen bond is seen in the X-ray structure of crystalline cellobiose[6], a compound being stereochemically identical with di-*N*-acetylchitobiose. The competitive, hydrogen-bond accepting properties of water lead us to expect such a hydrogen bond to be absent in aqueous solvents. On the other hand, it is quite likely that such a hydrogen bond could exist in F_6Pr-2-ol solution, as this solvent cannot act as a hydrogen-bond acceptor for OH-3 of the reducing, terminal residue.

The results just described suggest several relationships between c.d. spectra and oligosaccharide sequences having a 2-acetamido-2-deoxyhexose as the reducing terminus:

(a) The c.d. value at 209 nm for both water and F_6Pr-2-ol solutions of (1→6)-linked oligosaccharides is that of the free 2-acetamido-2-deoxy sugar in the same

solvent, a negative value for the water solution and a positive value for the F_6Pr-2-ol solution (see Figs. 1 and 2).

(b) For (1→4)-linked oligosaccharides, the c.d. value for the water solution is negative at 209 nm and positive at 190 nm, as observed for the chitin oligosaccharides by Coduti *et al.*[1]. For solutions in F_6Pr-2-ol, a negative c.d. value at 209 nm and 190 nm is observed (Fig. 5). The hypothesis that an intramolecular hydrogen-bond is responsible for the anomalous c.d. spectrum of di-*N*-acetylchitobiose in F_6Pr-2-ol solution implies that this observation is strictly true only for β-D-(1→4)-linked oligosaccharides, not for α-D-(1→4)-linked sequences.

(c) For (1→3)-linked disaccharides, a negative c.d. value at 204 nm is observed for water solutions, as well as for F_6Pr-2-ol solutions. The c.d. value for the π–π^* transition at 190 nm is negative or zero for both water and F_6Pr-2-ol solutions.

Additional predictions can be made about the dependence of the c.d. values, for F_6Pr-2-ol solutions, on the anomeric configuration of the interglycosidic linkage where a 2-acetamido-2-deoxyhexose is either at the nonreducing terminal position or is linked only at C-6. If it is linked in the α-D configuration, the c.d. value at 209 nm is positive ($[\theta] > 5000$) for the F_6Pr-2-ol solution, and if it is linked in the β-D configuration, the c.d. value is nearly zero ($[\theta] < 2000$).

It is possible that the rules just deduced could be useful in assigning structures to the oligosaccharide moiety of glycolipids or of oligosaccharides isolated from glycoproteins or polysaccharides. Since neutral sugars have no chromophores absorbing in the 190–220 nm region, the c.d. spectrum of oligosaccharides containing both neutral and acetamidodeoxy sugars is sensitive only to the linkages of the acetamidodeoxy residue. Such c.d. experiments are nondegradative and require ~300 nmoles of sample, free of contaminants absorbing in the 190–220 nm region.

EXPERIMENTAL

Materials. — The isolation and characterization of the disaccharides and derivatives used in this study have been reported previously[7].

Methods. — Commercially available samples of 2-acetamido-2-deoxy-D-glucose (**1**) and -D-galactose (**2**) were weighed, and their extinction coefficients were determined by measuring the u.v. absorption spectra on water and F_6Pr-2-ol solutions. Samples that were prepared in quantities too small to be weighed were dried and dissolved in either F_6Pr-2-ol or H_2O. The concentration of sugar in these solutions was determined from the absorption spectrum by use of the extinction coefficient of a parent sugar[1].

C.d. spectra were measured with a Cary 60 polarimeter equipped with a Model 6003 CD accessory. The spectra were electronically digitized, and the signals averaged and filtered by the Fourier method[8]. Absorption spectra were measured with a Cary 15 spectrometer under a flow of N_2.

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