Carbohydrate Research, 51 (1976) 263–268

Note

Circular dichroism studies of some oligosaccharides containing 2-acetamido-2-deoxy-D-glucopyranose and D-mannopyranose residues

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(Received February 2nd, 1976; accepted for publication in revised form, June 24th, 1976)

Cotton effects due to the amide chromophore in oligosaccharides were first reported by Beychok and Kabat[1] in 1965, and it has been shown that optical rotatory dispersion (o.r.d.) and circular dichroism (c.d.) measurements are of significant value in the elucidation of structure and sequence of oligo- and polysaccharides[1–5]. Cotton effects are observed in carbohydrates having the 2-acetamido chromophore, such a chromophore exhibiting two dichroic bands[6] at about 210 and 190 nm. By analogy with the well known peptide chromophore, these dichroic bands have been assigned to the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions, respectively. At these wavelengths, the D-mannopyranose residues do not show any dichroic band, and can be observed only in the tail of the far-ultraviolet c.d. band, which probably arises from an electronic transition in the ring oxygen[7].

The compounds investigated by o.r.d. and c.d. techniques include 2-acetamido-2-deoxyhexoses, milk oligosaccharides, and oligosaccharides derived from blood-group A, B, H, and Le^a substances[1–5]. A set of empirical rules related to the nature and position of the substituents of oligo- and polysaccharides containing 2-acetamido-2-deoxy-D-glucopyranosyl residues was proposed[4]. More recently, Dickinson and Bush[8] have shown that the dichroic spectra of *N*-acetylneuraminosyl-(2→3 and 2→6)-lactose are similar in shape but differ in magnitude; this result is additional evidence that the glycosidic linkages can be determined by use of the c.d. technique.

To date, no c.d. measurement has been performed on the oligosaccharides derived from the carbohydrate moiety of glycoproteins. The structure of some oligosaccharides has been elucidated and they were shown to contain a 2-acetamido-2-deoxy-D-glucopyranose residue[8]. In the present study, the c.d. spectra of oligosaccharides containing a 2-acetamido-2-deoxy-D-glucopyranose residue were examined in order to correlate the spectral variations with the structures of the oligosaccharides studied.

MATERIALS AND METHODS

Oligosaccharides. — Eight oligosaccharides (see Table I) were prepared from avian ovomucoid by partial acetolysis[10,11]: Four oligosaccharides (**1**, **2**, **3**, and **4**) contain the β-D-(1→4) linkage, two (**5** and **6**) the β-D-(1→2) linkage, and two (**7** and **8**) both β-D-(1→4) and -(1→2) linkages.

C.d. measurements. — The c.d. spectra were recorded with a Jobin–Yvon R.J. Mark III Dichrograph, at 24°, under a stream of high purity dry N_2, with a cell thickness of 0.01, 0.05, and 0.1 cm. Concentrations were adjusted to give absorbancies between 0.8 and 1.5. Total hexose content was determined by the orcinol–H_2SO_4 procedure[12–13] and hexosamine content by the Elson–Morgan reaction[14]. In all cases, the concentrations obtained from these two determinations were in full agreement with the structure of the oligosaccharide considered.

The molar ellipticity (θ) expressed in degree × cm^2 × $dmol^{-1}$ were calculated on the basis of the molar concentration of the amide group. The molecular weight of 2-acetamido-2-deoxy-D-glucose, D-mannose, and *O*-α-D-mannopyranosyl-(1→3)-D-mannose was taken as 221, 180, and 180, respectively. The results are reported in Table I. The ellipticity curves were constructed from at least five spectra.

TABLE I

ELLIPTICITY VALUES[a] AT THE EXTREMITIES OF THE C.D. SPECTRA OF SOME OLIGOSACCHARIDES CONTAINING A 2-ACETAMIDO-2-DEOXY-D-GLUCOPYRANOSE RESIDUE

Oligosaccharides	*Circular dichroism*	
	λ_{max} (*nm*)	$[\theta]_{max}$ (*degree* × cm^2 × $dmol^{-1}$)
O-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-D-mannose (**1**)	209	−4 850
	189	+35 850
O-α-D-Mannopyranosyl-(1→3)-*O*-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)]-D-mannose (**2**)	210	−6 400
	187	+25 500
O-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-*O*-α-D-mannopyranosyl-(1→3)-D-mannose (**3**)	210	−12 400
	189	+33 600
O-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-*O*-α-D-mannopyranosyl-(1→3)-*O*-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)]-D-mannose (**4**)	210	−7 100
	188	+22 750
O-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-D-mannose (**5**)	211	−11 350
	190	+28 600
O-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→3)-D-mannose (**6**)	209	−12 900
	186	+60 800
O-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-*O*-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)]-D-mannose (**7**)	209	−2 800
	189	+3 050
O-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-*O*-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)]-*O*-α-D-mannopyranosyl-(1→3)-*O*-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)]-D-mannose (**8**)	208	−4 000
	188	+9 200

[a]The ellipticities are given per amide residue.

RESULTS AND DISCUSSION

In the c.d. spectra (Fig. 1) of each component of the oligosaccharides studied, that of 2-acetamido-2-deoxy-D-glucose exhibits an $n \rightarrow \pi^*$ transition negative band at about 207 nm corresponding to the amide group chromophore[6]. In the 180–205 nm domain, only this band is observed. The lack of the expected c.d. band at ~190 nm corresponding to the $\pi \rightarrow \pi^*$ transition cannot be explained. It is of interest that both $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transition c.d. bands were observed by Stone[6] with 2-acetamido-2-deoxy-D-galactose. As expected, the c.d. spectra of D-mannose and *O*-α-D-mannopyranosyl-(1→3)-D-mannose do not exhibit these bands; only the tail of a weak, wavelength-positive band was observed.

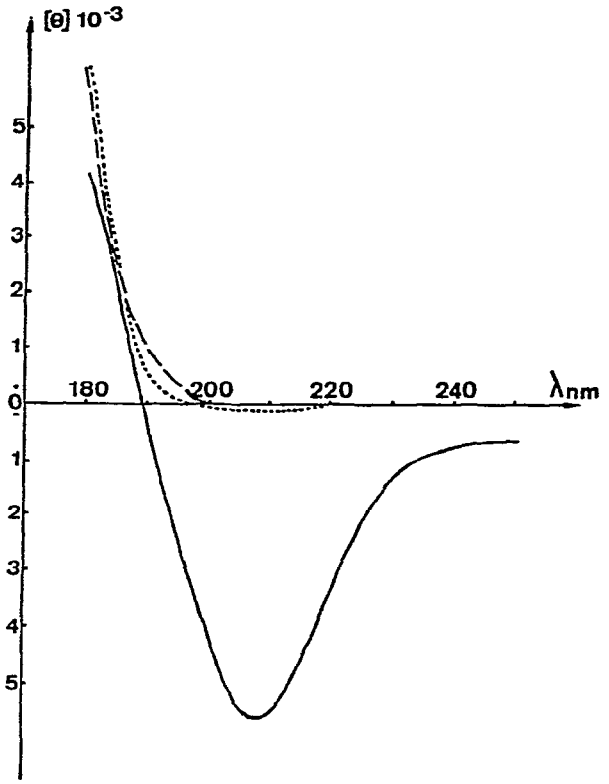

Fig. 1. Circular dichroism spectra of 2-acetamido-2-deoxy-D-glucose (———), D-mannose (— — —) and *O*-α-D-mannopyranosyl-(1→3)-D-mannose (· · ·). Ellipticities in degree × cm² × dmol⁻¹ are given per amide residue for the first compound, per molecule for the two other compounds.

The c.d. spectra of **1**, **2**, **3**, and **4** having a β-D-(1→4) linkage (see Fig. 2) show quite similar curve shapes, and exhibit a negative band at ~210 nm and a positive band at ~189 nm due to the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions, respectively, of the amide group chromophore. A wide variation in the magnitude of the c.d. is observed,

however, even for similar oligomers. For example, **2** and **3** present the same composition and the same linkages, but differ only in the sequence of the components, **2** being a branched oligosaccharide (see structures in Table I). Since the shapes of the c.d. spectra of **2** and **3** are the same, the distinction in magnitude of both spectra is a reliable indication only if the molar ellipticities (hence the concentrations) are known accurately. Since both determinations of the concentrations gave results in excellent agreement, it can be concluded that the difference observed in the c.d. spectra is due to the different structures.

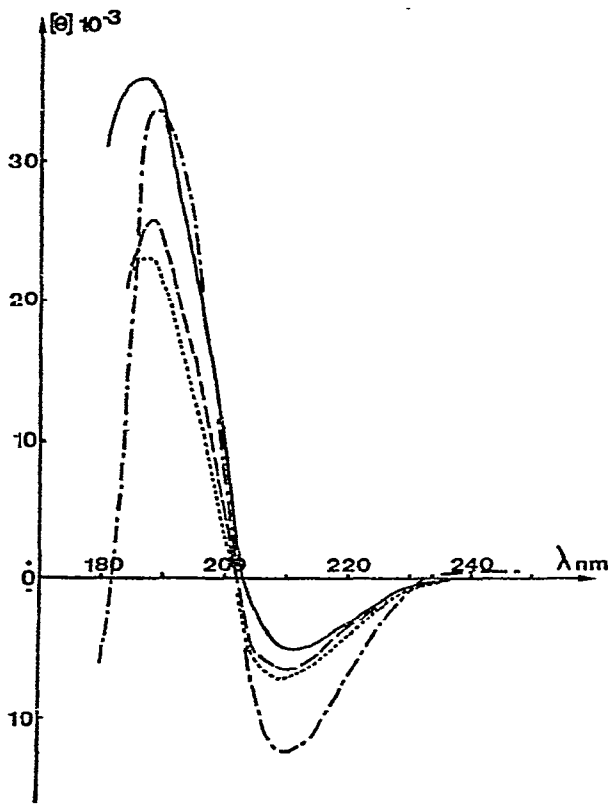

Fig. 2. Circular dichroism spectra of *O*-2-acetamido-2-deoxy-*β*-D-glucopyranosyl-(1→4)-D-mannose (**1**) (———), *O*-α-D-mannopyranosyl-(1→3)-*O*-[2-acetamido-2-deoxy-*β*-D-glucopyranosyl-(1→4)]-D-mannose (**2**) (— — —), *O*-2-acetamido-2-deoxy-*β*-D-glucopyranosyl-(1→4)-*O*-α-D-mannopyranosyl-(1→3)-D-mannose (**3**) (— · —·), and *O*-2-acetamido-2-deoxy-*β*-D-glucopyranosyl-(1→4)-*O*-α-D-mannopyranosyl-(1→3)-*O*-[2-acetamido-2-deoxy-*β*-D-glucopyranosyl-(1→4)]-D-mannose (**4**) (· · ·). Ellipticities in degree × cm^2 × $dmol^{-1}$ are given per amide residue.

Compounds **1** and **3** only differ by an additional D-mannose residue, which does not possess an amide group chromophore c.d. band, and two identical c.d. curves were expected. However, as shown in Fig. 2, the spectra of **1** and **3** exhibit an

unexpectedly wide difference in amplitude, the amplitude of the ellipticity of **3** being greater than that of **1**.

In **4**, one of the two 2-acetamido-2-deoxy-β-D-glucopyranosyl residues is linked (1→4) branchwise, as for **2**, and the other residue linearly, as for **3**. The c.d. curve (Fig. 2) shows that no simple linear combination between the ellipticities of **2** and **3**, and that of **4** exists since the amplitude of the c.d. bands of **4** is slightly more intense than that of **2**.

The c.d. spectra of **5**, **6**, **7**, and **8** (see Fig. 3) are similar to those obtained previously and some variations of the amplitude of the c.d. spectra is also observed. Compounds **5** and **6** have a linear structure and differ only by an additional D-mannose residue. As shown in Fig. 3, the ellipticity of **6** is greater than that of **5**, as expected from the results of **1** and **3**.

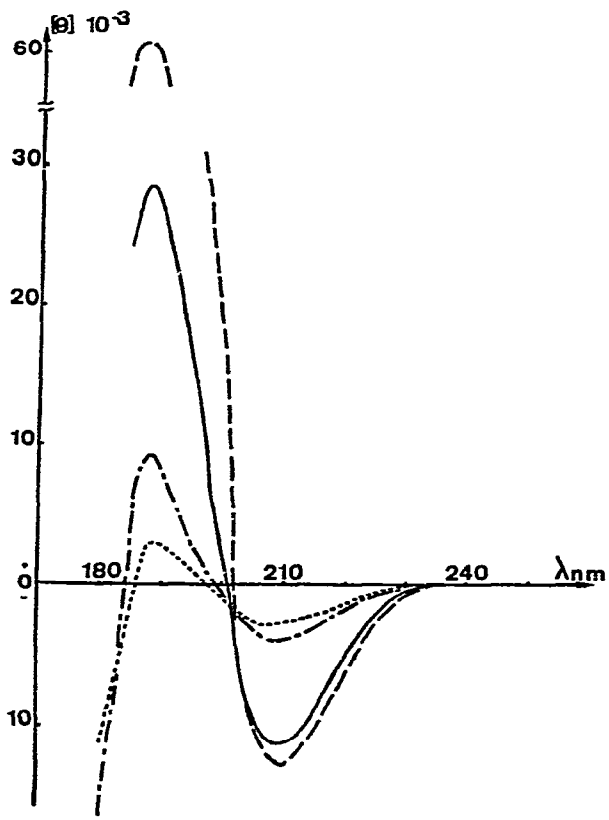

Fig. 3. Circular dichroism spectra of *O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-D-mannose (**5**) (———), *O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→3)-D-mannose (**6**) (— — —), *O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-*O*-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)]-D-mannose (**7**) (· · ·), and *O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-*O*-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)]-*O*-α-D-mannopyranosyl-(1→3)-*O*-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)]-D-mannose (**8**) (— · — · —). Ellipticities are expressed as in Fig. 2.

From the c.d. spectra of **1**, **3**, **5**, and **6** (Figs. 2 and 3), it can be concluded that the nature of the linkage between 2-acetamido-2-deoxy-D-glucose and D-mannose residues affects the magnitude of the c.d. bands. Indeed, the only difference between **1** and **5**, which do not present the same ellipticities, is the fact that this linkage is β-D-(1→4) for **1** but β-D-(1→2) for **5**. In a similar manner, it can be observed that the c.d. curves of **3** and **6**, in which the linkage is β-D-(1→4) for **3** and β-D-(1→2) for **6**, do not show the same magnitude for their ellipticities.

We have attempted to establish, for **1**, **3**, **5** and **6**, a correlation between the ellipticities of each compound and that of their components, but without success.

Actually, the c.d. spectra of **1** and **5** do not coincide with the spectrum calculated from a linear combination of the c.d. spectra of 2-acetamido-2-deoxy-D-glucose and D-mannose. To date, an interpretation of the c.d. spectra of **7** and **8** is not possible.

From these results, it is easy to observe a distinction in the c.d. spectrum corresponding to the β-D-(1→4) and the β-D-(1→2) linkages of the 2-acetamido-2-deoxy-D-glucopyranose residues. Thus, the c.d. curves seem to be a good tool for the determination of the sequence and of the location of the *O*-glycosyl bonds of the various components of the eight oligosaccharides studied here. Similar studies may be performed with other oligosaccharides derived from the carbohydrate moiety of glycoproteins, at the condition that they contain 2-acetamido-2-deoxy-D-glucopyranose, 2-acetamido-2-deoxy-D-galactopyranose, or *N*-acetylneuraminic acid residues that give a c.d. signal in the wavelength region used in this work.

ACKNOWLEDGMENTS

We are indebted to Professor G. Biserte for helpful discussions that were extremely valuable to us and we thank Mrs. M. P. Hildebrand for her excellent technical assistance.

REFERENCES

1 S. BEYCHOK AND E. A. KABAT, *Biochemistry*, 4 (1965) 2565–2574.
2 K. O. LLOYD, S. BEYCHOK, AND E. A. KABAT, *Biochemistry*, 6 (1967) 1448–1454.
3 K. O. LLOYD, S. BEYCHOK, AND E. A. KABAT, *Biochemistry*, 7 (1968) 3762–3765.
4 E. A. KABAT, K. O. LLOYD, AND S. BEYCHOK, *Biochemistry*, 8 (1969) 747–756.
5 K. O. LLOYD, E. A. KABAT, AND S. BEYCHOK, *J. Immunol.*, 102 (1969) 1354–1358.
6 A. L. STONE, *Biopolymers*, 10 (1971) 739–751.
7 L. W. PICKETT, N. J. HOEFLICK, AND T. C. LIU, *J. Am. Chem. Soc.*, 73 (1951) 4865–4869.
8 H. R. DICKINSON AND C. A. BUSH, *Biochemistry*, 14 (1975) 2299–2304.
9 J. MONTREUIL, *Pure Appl. Chem.*, 42 (1975) 431–477.
10 B. BAYARD AND J. MONTREUIL, *Carbohydr. Res.*, 24 (1972) 427–433.
11 B. BAYARD, B. FOURNET, S. BOUQUELET, G. STRECKER, G. SPIK, AND J. MONTREUIL, *Carbohydr. Res.*, 24 (1972) 445–456.
12 J. TILLMANS AND K. PHILIPPI, *Biochem. Z.*, 215 (1929) 36–60.
13 C. RIMINGTON, *Biochem. J.*, 25 (1931) 1062–1067; *ibid.*, 34 (1940) 931–940.
14 R. BELCHER, A. J. NUTTEN, AND C. M. SAMBROOK, *Analyst (London)*, (1954) 201–208.