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Carbohydrate Research 322 (1999) 135–141

CARBOHYDRATE RESEARCH

Acetate groups as probes of the stereochemistry of carbohydrates: a vibrational circular dichroism study

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Received 26 May 1999; accepted 4 August 1999

Abstract

Vibrational absorption and circular dichroism spectra of five different monosaccharide pentaacetates were measured. These spectra were found to be dominated by the contributions from the acetate groups. In the carbonyl region, α- and β-D-glucose pentaacetates do not show measurable vibrational circular dichroism (VCD), but α- and β-D-galactose pentaacetates and α-D-mannose pentaacetate show significant VCD, with mannose pentaacetate giving opposite signs to those of α- and β-D-galactose pentaacetates. These observed features are interpreted to reflect the orientation of carbonyl groups around the carbohydrate ring. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Carbohydrates; Monosaccharides; Pentaacetates; Vibrational circular dichroism; Infrared spectra; Vibrational spectra; Circular dichroism

1. Introduction

Vibrational circular dichroism (VCD) [1] measures the differential absorption of left versus right circularly polarized IR light originating from vibrational transitions of chiral molecules. All $(3N-6)$ vibrational modes of a chiral molecule, N being the number of atoms in the molecule, can give rise to circular dichroism. This is an advantage compared with electronic circular dichroism (ECD) [2], where only a limited number of electronic transitions are usually accessible. Owing to such large number of available transitions, VCD can lead to much more detailed stereochemical information on the molecule of interest. This becomes all the more important for simple carbohydrates, which do not have electronic transitions in the visible range, and the ultraviolet region is more difficult to access. Vibrational Raman optical activity (VROA) [3], which is the Raman counterpart of VCD, has been shown [4–11] to be a very useful tool for studying carbohydrates. The complicated nature of vibrational modes and overlapping vibrational bands generally pose problems in the interpretation of vibrational optical activity spectra. Nevertheless, qualitative trends in the observed VROA spectra have provided useful applications ranging from monosaccharides to polysaccharides [4–11].

The smaller VCD signals seen for carbohydrates, compared with those seen for molecules with rigid structures, have limited the number of VCD studies in the past on carbohydrates. Improvements in VCD instrumentation have now led to better quality

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PII: S0008-6215(99)00211-6

VCD measurements with enhanced signal-to-noise ratios, so carbohydrates can now be investigated. Previous VCD studies on carbohydrates include four papers in the mid-IR region [12–14] and two papers in the hydrogen-stretching region [15,16]. Unsubstituted monosaccharides generally exhibit broad absorption and weak VCD features in the mid-IR region, making it relatively difficult to deduce the stereochemistry of monosaccharides. This problem may be overcome when hydroxyl groups are substituted with functional groups. To investigate this possibility, we studied the role of acetate groups on the VCD spectra of monosaccharides using the state-of-the-art instrumentation in the $\sim 2000–1120\ cm^{-1}$ region.

2. Experimental

The chemicals were used as received from Sigma Chemical Co. Since the pentaacetates studied here have very low solubilities in water, dimethyl sulfoxide-d_6 (Me_2SO-d_6) was used as the solvent. Me_2SO-d_6 was purchased from Cambridge Isotopes Laboratory. The IR and VCD spectra were recorded on a commercial Fourier-transform VCD spectrometer, Chiralir (Bomem-BioTools, Canada) with a ZnSe beamsplitter, BaF_2 polarizer, optical filter (transmitting below $2000\ cm^{-1}$) and a 2×2 mm HgCdTe detector. One difference from the standard Chiralir instrument is that the photoelastic modulator used was a PEM-80 model (Hinds Instruments) without AR coating on the ZnSe optical element. The VCD spectra were recorded, using the supplied Chiralir software, with 1 h data collection time at $4\ cm^{-1}$ resolution.

The transmission properties of optical filter and BaF_2 substrates used in the instrument restrict the range of measurements to 2000–$900\ cm^{-1}$. The strong absorption of Me_2SO-d_6 solvent precludes observing VCD features in the $\sim 1120–900\ cm^{-1}$ region. The absorption and VCD spectra of Me_2SO-d_6 solvent obtained under identical conditions were subtracted out from those of samples dissolved in Me_2SO-d_6 solvent.

3. Results and discussion

The vibrational absorption spectra of unsubstituted monosaccharides in the 1600–1200 cm^{-1} region are usually broad and weak. However, in the case of pentaacetates, the absorption spectra are well resolved and have strong bands. For a given concentration and pathlength, the absorption intensities are approximately ten times larger in monosaccharide pentaacetates than those in the corresponding monosaccharides (see Fig. 1). These observations suggest that the spectra of monosaccharide pentaacetates are dominated by the absorption bands arising from the vibrational modes of the acetate groups. The acetate group vibrational modes can be divided into three regions; (a) carbonyl modes appearing in the 1800–1600 cm^{-1} region; (b) methyl group modes appearing in the 1500–1300 cm^{-1} region and (c) C–O/C–C stretching modes appearing in the 1300–1100 cm^{-1} region. The bands that exhibit measurable VCD are summarized in Table 1.

Carbonyl group modes (1800–1600 cm^{-1} region).—The VCD spectra of the five pentaacetates in the carbonyl region (1800–1600 cm^{-1}) are displayed in Fig. 2. For α- and β-D-glucose pentaacetates, the carbonyl absorption maxima (see Table 1) are, respectively, at 1753 and 1757 cm^{-1} (with a clearly resolved shoulder for the latter at 1745 cm^{-1}), while for α-D-galactose, β-D-galactose, and α-D-mannose pentaacetates the corresponding maxima are at 1751 cm^{-1}. The VCD associated with α- and β-D-glucose pentaacetates is weak and is within the noise level of the measurement. Conservative *positive* VCD couplets (a bisignate VCD with positive VCD on the lower-frequency side and negative VCD on the higher-frequency side), with a slight negative bias, are seen for α- and β-D-galactose pentaacetates. However, the magnitude of the VCD couplet is larger in α-D-galactose pentaacetate ($\Delta A/A = \sim 10^{-4}$) than in β-D-galactose pentaacetate ($\Delta A/A = \sim 4 \times 10^{-5}$). In the case of α-D-mannose pentaacetate, a *negative* VCD couplet, with a slight negative bias, is seen ($\Delta A/A = \sim 10^{-4}$). The observation in galactose pentaacetates that the α anomer has larger VCD signals than the β

anomer, was also seen earlier [14] for α- and β-D-glucose in Me_2SO-d_6 and for α- and β-linked disaccharides in aqueous solutions.

The observation that α- and β-D-galactose pentaacetates show the same signed VCD couplet, while α-D-mannose pentaacetate shows an oppositely signed couplet, must be related to the stereochemical arrangement of the carbonyl groups around the carbohydrate ring. To explore this stereochemical relation, we have assembled the structures of these five pentaacetates using the ChemSite program [17] and have optimized these structures with a built-in optimization algorithm. The dihedral angles between the carbonyl groups located at successive ring carbon atoms were then derived from these optimized structures (see Table 2). Assuming that each pair of

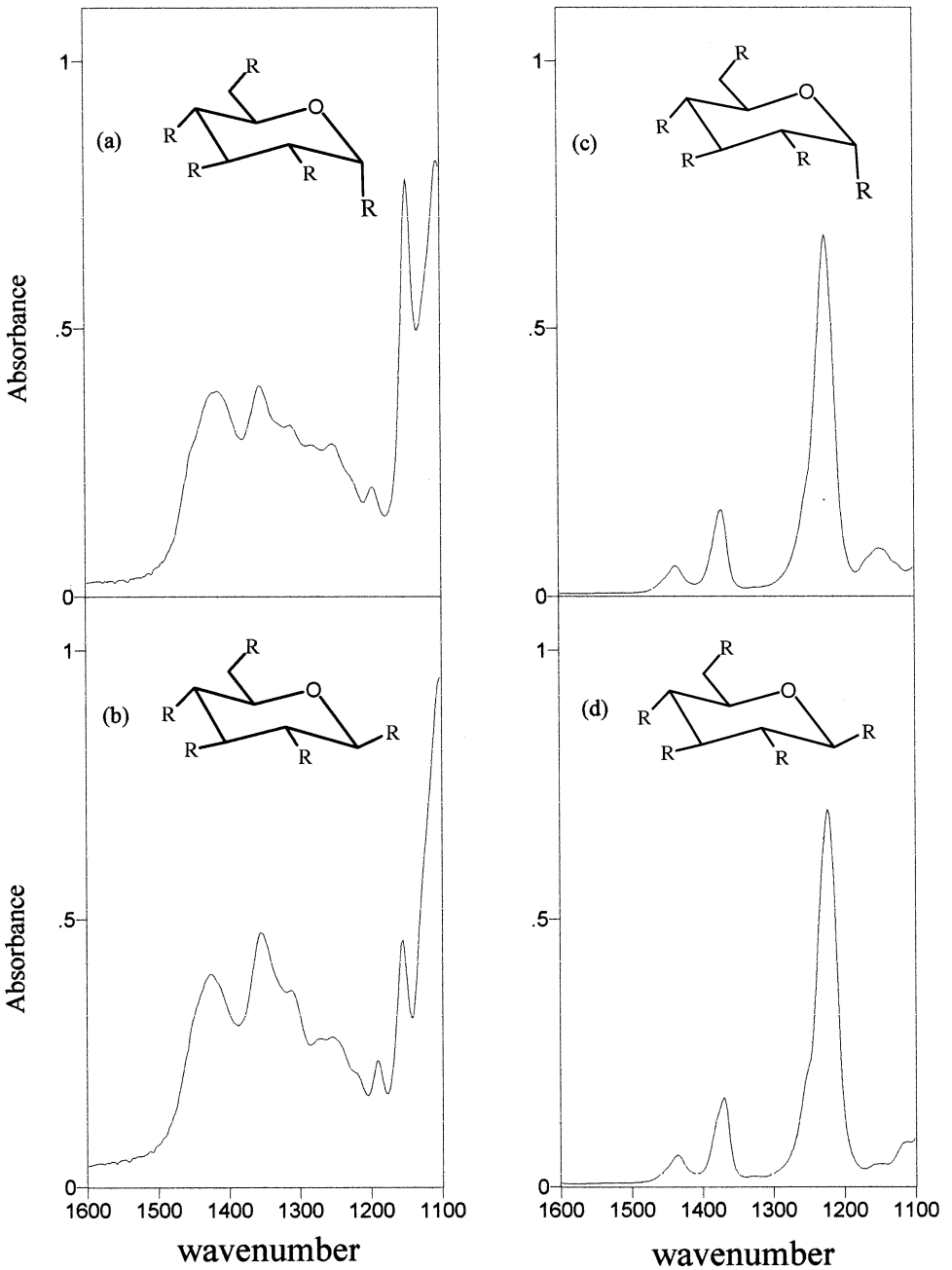

Fig. 1. Vibrational absorption spectra of monosaccharides and their pentaacetates, in the 1600–1100 cm^{-1} range, in Me_2SO-d_6 solutions. (a) α-D-Glucose (0.5 M, 70 μm pathlength); (b) β-D-glucose (0.5 M, 70 μm pathlength); (c) α-D-glucose pentaacetate (0.1 M, 33 μm pathlength); (d) β-D-glucose pentaacetate (0.1 M, 33 μm pathlength). In the structures displayed, R = OH for monosaccharides and R = $OCOCH_3$ for pentaacetates.

Table 1
Some absorption and VCD bands of monosaccharide pentaacetates

-Pentaacetate	Carbonyl group		Methyl group		C–O/C–C group	
	Absorption [a]	VCD [a] ($\Delta A/A$) [b]	Absorption [a]	VCD [a] ($\Delta A/A$) [b]	Absorption [a]	VCD [a] ($\Delta A/A$) [b]
α-D-Glucose-	1753		1371	1391 (−16) 1377 (+8)	1225	1227 (−6) 1211 (+7)
β-D-Glucose-	1757		1369	1389 (−5) 1369 (−4)	1223	1227 (−5) 1210 (+15)
α-D-Galactose-	1751	1755 (−12) 1743 (+12)	1374	1394 (−21) 1375 (+7)	1228	1250 (+11) 1229 (−12) 1215 (+5)
β-D-Galactose-	1751	1753 (−4) 1738 (+8)	1371	1387 (−11) 1373 (+7)	1225	1248 (+4) 1227 (−12) 1215 (+6)
α-D-Mannose-	1751	1759 (+12) 1745 (−11)	1372	1395 (+17) 1369 (−7)	1223	1255 (−12) 1227 (1) 1214 (−5)

[a] Band position (in cm^{-1}).
[b] Dissymmetry factor (in 10^{-5} units).

carbonyl groups at successive ring carbon atoms behaves as a coupled oscillator, we simulated the VCD spectra using the coupled oscillator model [18]; however, the predicted spectra did not resemble the observed spectra. Then we assumed that the overall carbonyl VCD contribution for each pentaacetate may be reflected by the average of dihedral angles of the oscillators, although a theoretical basis for this assumption is yet to be formulated. The average dihedral angle of C=O oscillators is close to zero in α- and β-D-glucose pentaacetates (Table 2), it is of larger magnitude with the same sign in α- and β-D-galactose pentaacetates and it is of larger magnitude but with opposite sign in α-D-mannose pentaacetate. This pattern matches the observed carbonyl VCD sign patterns (although the dihedral angles alone cannot explain the lower VCD magnitudes observed for β anomer). In this manner, the carbonyl VCD data of the five pentaacetates studied here may be interpreted to reflect the overall stereochemistry of the carbohydrate ring.

Methyl group modes (1500–1300 cm^{-1} region).—The characteristic vibrations of the methyl group are two antisymmetric bending modes and a symmetric bending mode. The former appear at about 1435 cm^{-1} and exhibit no measurable VCD. The symmetric bending mode, appearing in the range $\sim$1370–1374 cm^{-1}, however, exhibits measurable VCD for all of the pentaacetates (see Table 1 and Fig. 3). A *positive* VCD couplet is present in α-D-glucose, α-D-galactose and β-D-galactose pentaacetates, and a *negative* VCD couplet is present in α-D-mannose pentaacetate. In the case of β-D-glucose pentaacetate, two negative VCD bands are seen to be associated with the absorption band at 1369 cm^{-1}. The VCD sign reversal from α- and β-D-galactose pentaacetates to α-D-mannose pentaacetate may be explained in a manner analogous to that used for carbonyl group modes, but significant change in pattern from α-D-glucose pentaacetate to β-D-glucose pentaacetate suggests that some coupling of methyl group modes with, for example, the methine group modes might be involved. Then a simple criterion is not apparent for relating the stereochemistry of carbohydrates with the observed VCD of methyl group modes.

C–O/C–C stretch group modes (1300–1100 cm^{-1} region).—The intense absorption band seen in the range of 1223–1228 cm^{-1} for each of the pentaacetates (see Fig. 1) is considered to be arising from the C–O or C–C stretching modes of the acetate groups. For these modes a *positive* VCD couplet is present in α- and β-D-glucose pentaacetates (see Table 1). But in α- and β-D-galactose pentaacetates, a positive–negative–positive triplet pattern is seen with the negative band being stronger than the two positive bands (see Table 1). This sign

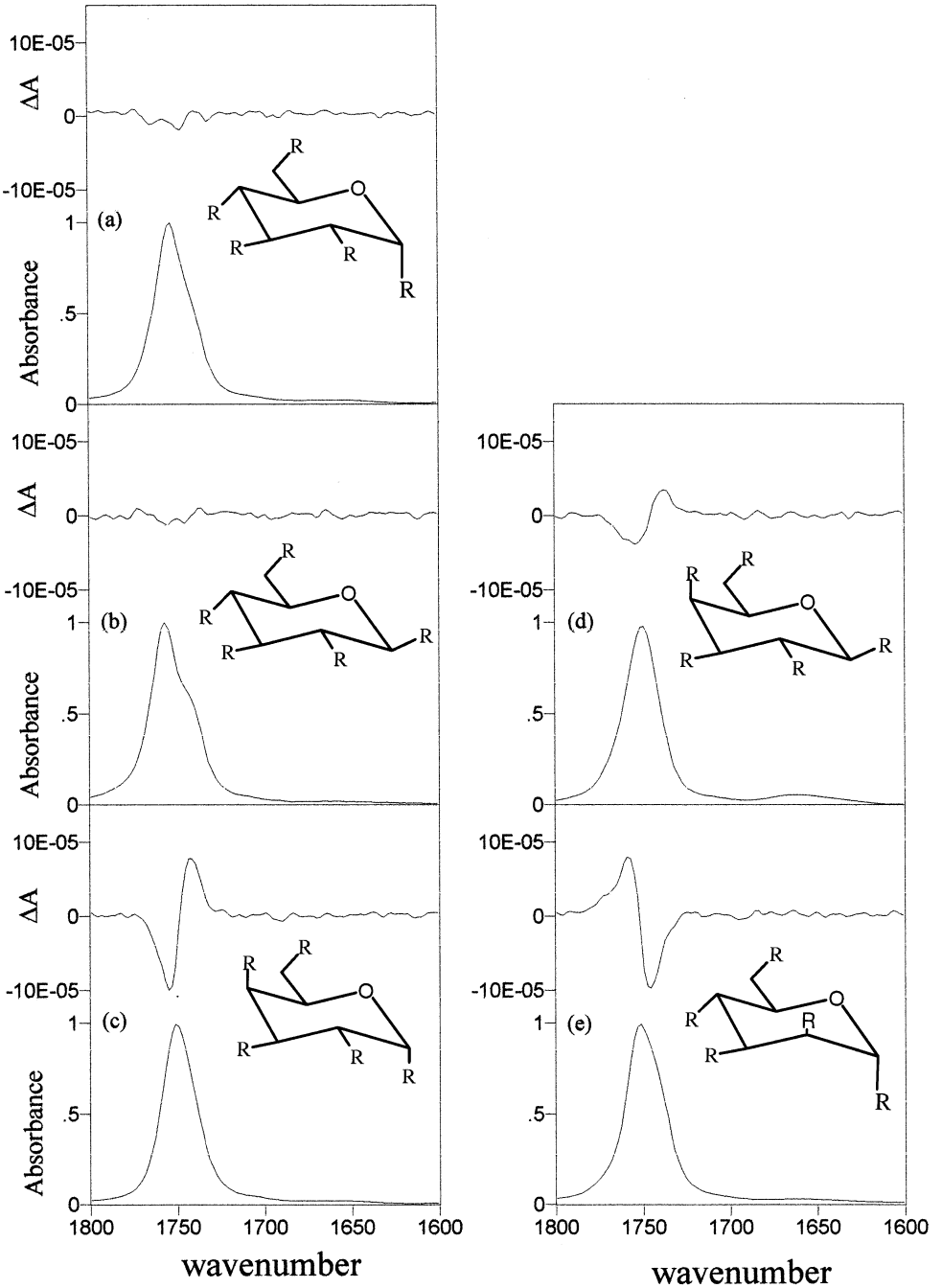

Fig. 2. Vibrational absorption (bottom) and VCD (top) spectra, in the 1800–1600 cm^{-1} region, in Me_2SO-d_6 solutions of monosaccharide pentaacetates. (a) α-D-Glucose pentaacetate; (b) β-D-glucose pentaacetate; (c) α-D-galactose pentaacetate; (d) β-D-galactose pentaacetate; (e) α-D-mannose pentaacetate. In all five cases the concentration and pathlength used were 0.1 M and 33 μm, respectively. Under these conditions, the peak absorbances in these five cases were 0.67, 0.61, 0.66, 0.67, and 0.64, respectively. The absorption and VCD displayed are scaled to a maximum absorbance of 1. In the structures displayed, $R = OCOCH_3$.

pattern is reversed in α-D-mannose pentaacetate (see Table 1). Because of the presence of a VCD triplet, a simple model does not seem to be applicable for these modes also.

4. Summary

Among the three different types of modes associated with the acetate groups, the sym-

Table 2
Orientations of carbonyl groups around the carbohydrate ring

Name	Dihedral angles (°) [a]				
	C-1–C-2	C-2–C-3	C-3–C-4	C-4–C-5	Average
α-D-Glucose pentaacetate	−35.1	+172.4	−166.7	−4.5	−8.5
β-D-Glucose pentaacetate	+32.1	+172.1	−171.3	−53.2	−5.1
α-D-Galactose pentaacetate	−37.3	+163.1	+28.2	−13.1	+35.2
β-D-Galactose pentaacetate	+28.9	+162.9	+15.9	−8.5	+49.8
α-D-Mannose pentaacetate	+172.8	−110.9	−178.8	−135.3	−63.1

[a] Dihedral angles are for OC···CO segments at adjacent carbons of the carbohydrate ring.

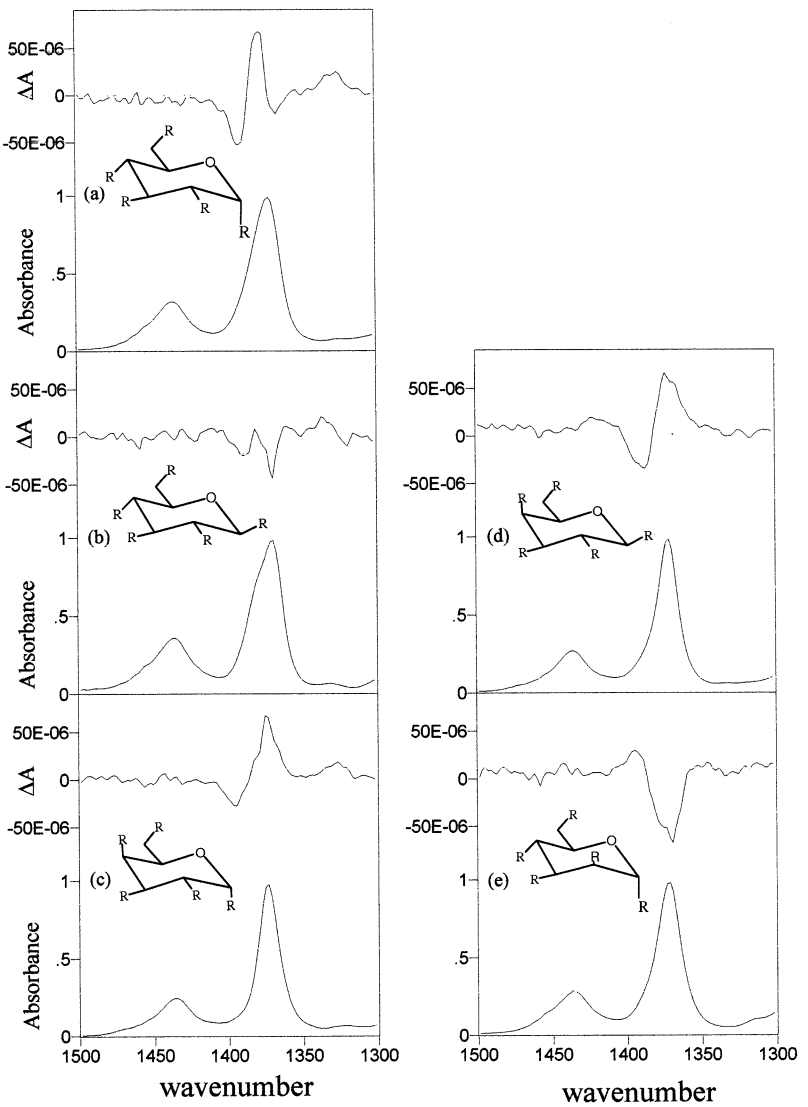

Fig. 3. Vibrational absorption (bottom) and VCD (top) spectra, in the 1500–1300 cm^{-1} region, in Me_2SO-d_6 solutions of monosaccharide pentaacetates. (a) α-D-Glucose pentaacetate (0.5 M, 30 μm pathlength); (b) β-D-glucose pentaacetate (0.1 M, 150 μm pathlength); (c) α-D-galactose pentaacetate (0.5 M, 28 μm pathlength); (d) β-D-galactose pentaacetate (0.1 M, 113 μm pathlength); (e) α-D-mannose pentaacetate (0.5 M, 23 μm pathlength). The peak absorbances in these fives cases were 0.87, 0.63, 0.69, 0.62, and 0.57, respectively. The absorption and VCD displayed are scaled to a maximum absorbance of 1. In the structures displayed, R = $OCOCH_3$.

metric methyl bending mode and C–O/C–C stretching modes appear to involve coupling with other vibrations, making them less useful for stereochemical deductions. However, the carbonyl stretching modes, which are not usually coupled to other vibrations, appear to provide a simple correlation between the observed VCD and the stereochemistry of carbohydrates, although a theoretical basis for this correlation is yet to be found. The carbonyl stretching VCD might serve as a useful stereochemical probe for carbohydrates, just as it did for proteins [19].

Acknowledgements

A grant from NSF (CHE9707773) and Vanderbilt University are gratefully acknowledged for support. We thank Dr Max Diem for providing his computer program that simulates VCD using the extended coupled oscillator model.

References

[1] (a) F. Long, T.B. Freedman, R. Hapanowicz, L.A. Nafie, *Appl. Spectrosc.*, 51 (1997) 504–507. (b) F.J. Devlin, P.J. Stephens, J.R. Cheesman, M.J. Frisch, *J. Am. Chem. Soc.*, 118 (1996) 6327–6328. (c) P. Bour, C.N. Tam, M. Shaharuzzaman, J.S. Chicos, T.A. Keiderling, *J. Phys. Chem.*, 37 (1996) 15041–15048. (d) A. Rauk, D. Yang, D. Tsankov, H. Wieser, Yu. Koltypin, A. Gedanken, G.V. Shustov, *J. Am. Chem. Soc.*, 117 (1995) 4160–4166. (e) H.R. Wysbrod, M. Diem, *Biopolymers*, 32 (1992) 1237–1242. (f) P.L. Polavarapu, A. Cholli, G.J. Vernice, *J. Am. Chem. Soc.*, 114 (1992) 10953–10955.

[2] K. Nakanishi, N. Berova, R.W. Woody, *Circular Dichroism: Principles and Applications*, VCH, New York, 1994.

[3] (a) J. Costante, L. Hecht, P.L. Polavarapu, A. Collet, L.D. Barron, *Angew. Chem., Int. Ed. Engl.*, 36 (1997) 885–887. (b) C.N. Tam, P. Bour, T.A. Keiderling, *J. Am. Chem. Soc.*, 118 (1996) 10285–10293. (c) G.-S. Yu, D. Che, T.B. Freedman, L.A. Nafie, *Biospectroscopy*, 1 (1995) 113–123.

[4] L.D. Barron, A.R. Gargaro, Z.Q. Wen, D.D. MacNicol, C. Butters, *Tetrahedron: Asymmetry*, 1 (1990) 513–516.

[5] L.D. Barron, A.R. Gargaro, Z.Q. Wen, *Carbohydr. Res.*, 210 (1991) 39–40.

[6] Z.Q. Wen, L.D. Barron, L. Hecht, *J. Am. Chem. Soc.*, 115 (1993) 285–292.

[7] A.F. Bell, L.D. Barron, L. Hecht, *Carbohydr. Res.*, 257 (1994) 11–24.

[8] A.F. Bell, L. Hecht, L.D. Barron, *J. Raman Spectrosc.*, 24 (1993) 633–635.

[9] A.F. Bell, L. Hecht, L.D. Barron, *J. Am. Chem. Soc.*, 116 (1994) 5155–5161.

[10] A.F. Bell, L. Hecht, L.D. Barron, *Spectrochim. Acta*, 51 (1995) 1367–1378.

[11] A.F. Bell, L. Hecht, L.D. Barron, *J. Raman Spectrosc.*, 26 (1995) 1071–1074.

[12] D.M. Back, P.L. Polavarapu, *Carbohydr. Res.*, 133 (1984) 163–167.

[13] C.M. Tummalapalli, D.M. Back, P.L. Polavarapu, *J. Chem. Soc., Faraday Trans.*, 84 (1988) 2585–2594.

[14] (a) P.K. Bose, P.L. Polavarapu, *Carbohydr. Res.*, 319 (1999) 172–183. (b) P.K. Bose, P.L. Polavarapu, *J. Am. Chem. Soc.*, 121 (1999) 6094–6095.

[15] C. Marcott, H.A. Havel, J. Overend, A. Moscowitz, *J. Am. Chem. Soc.*, 100 (1978) 7088–7089.

[16] M.G. Paterlini, T.B. Freedman, L.A. Nafie, *J. Am. Chem. Soc.*, 108 (1986) 1389–1397.

[17] ChemSite for Windows, Pyramid Learning, Aptos, CA 95001, USA, 1998.

[18] (a) I. Tinoco Jr., *Rad. Res.*, 20 (1963) 133–139. (b) G. Holzwarth, I. Chabay, *J. Chem. Phys.*, 57 (1972) 1632–1635. (c) L.D. Barron, *Molecular Light Scattering and Optical Activity*, Cambridge University Press, Cambridge, 1982. (d) M. Gulotta, D.J. Goss, M. Diem, *Biopolymers* 28 (1989) 2047–2058.

[19] V. Baumruk, T.A. Keiderling, *J. Am. Chem. Soc.*, 115 (1993) 6939–6940.