centrated solutions of **1** it may be responsible for the autocatalytic chain decomposition reaction discovered by Waddell and coworkers.[30] In highly dilute solutions or viscous media the formation of **3** may be reversible and eventually give rise to a delayed formation of ^{3}**2**.[11,27]

(30) Costantino, J. P.; Richter, H. W.; Lee Go, C. H.; Waddell, W. H. *J. Am. Chem. Soc.* **1985**, 1744-1747.

Acknowledgment. This work was supported by a USNSF (international division) travel grant to M.S.P. and by the Swiss National Science Foundation, Project 2.213-0.84. Financial support was received from Ciba-Geigy SA, Hoffmann-La Roche SA, Sandoz, SA, and the Ciba-Stiftung.

Registry No. 1, 622-37-7; **2**, 2655-25-6; **7**, 1423-15-0; **8**, 26334-20-3; **9**, 102284-85-5; **10**, 13125-57-0.

Vacuum Ultraviolet Circular Dichroism of Dermatan Sulfate: Iduronate Ring Geometry in Solution and Solid State

David G. Cziner,[†] Eugene S. Stevens,*[†] Edwin R. Morris,[‡] and David A. Rees[§]

Contribution from the Department of Chemistry, State University of New York, Binghamton, New York 13901, Department of Food Research and Technology, Cranfield Institute of Technology, Silsoe College, Silsoe, Bedford MK45 4DT, U.K., and National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K. *Received June 7, 1985*

Abstract: The circular dichroism of dermatan sulfate was measured to 170 nm in solution and 155 nm in films. A previously unobserved CD band is found near 175 nm. The CD spectra of solutions and films are essentially identical, indicating no significant conformational change on drying. A quadrant rule for the 175-nm band in polysaccharides is proposed that rationalizes the large data bank of CD spectra in this wavelength region. The positive sign of the 175-nm band in dermatan sulfate indicates that the $^1C_4$ iduronate ring conformation is predominant in both solution and films.

Glycosaminoglycans are components of animal connective tissue found mainly in the extracellular matrix. They are unbranched polysaccharide chains composed of repeating disaccharide units. In the hyaluronate–chondroitin–dermatan family of glycosaminoglycans, the disaccharide unit consists of a hexosamine and a uronic acid, with glycosidic linkages alternately (1→3) and (1→4). The hexosamine in both chondroitins and dermatans is 2-acetamido-2-deoxy-β-D-galactose (β-D-GalNAc). The uronic acid in chondroitins is β-D-glucuronic acid (β-D-GlcA); in dermatans it is α-L-iduronic acid (α-L-IdA). The hexosamine occurs sulfated at the 4-position in chondroitin 4-sulfate (chondroitin A) and dermatan sulfate (chondroitin B), and at the 6-position in chondroitin 6-sulfate (chondroitin C). Structural heterogeneity exists such that the degree of sulfation is variable, and in dermatans, β-D-GlcA occurs to varying extents as a minor component, partially replacing α-L-IdA. The primary structures, available crystallographic data, and biological functions of glycosaminoglycans have recently been reviewed.[1] Figure 1 shows the predominant repeating disaccharide unit of dermatan sulfate.

In the $^4C_1$ ring conformation of β-D-GlcA residues in chondroitins, the linkage oxygens [O(1), O(4)], the hydroxyl groups at O(2) and O(3), and the carboxylate substituents at C(6) all occupy equatorial locations, well separated from each other around the periphery of the sugar ring, giving rise to a particularly stable chair geometry. On C(5) epimerization to α-L-Ida (as in dermatan sulfate), the $^4C_1$ conformation is destabilized by placing the bulky carboxylate group in a sterically crowded axial position. C(6) can be shifted to an equatorial location by ring inversion to the $^1C_4$ chair conformation, but this is accompanied by conversion of the equatorial arrangement of the other four substituents [O(1), O(2), O(3), O(4)] to axial, which again induces severe steric crowding. Thus, both chair forms of α-L-IdA are far less stable than the $^4C_1$ conformation of β-D-GlcA, and the conformational preference of the ring is delicately balanced between them. The conformation of iduronate residues in dermatan sulfate is, in particular, an area of current controversy.

Studies of conformational equilibria in model idopyranosides favor the $^1C_4$ form [C(6) equatorial],[2] and the proton NMR of dermatan sulfate solutions also indicates adoption of this ring geometry.[3] The susceptibility of dermatan sulfate solutions to periodate oxidation,[4] however, has been interpreted as indicating a predominance of the $^4C_1$ form in which the hydroxyl groups at C(2) and C(3) are trans-diequatorial, rather than trans-diaxial as in the $^1C_4$ conformation indicated by NMR. This apparent discrepancy can be resolved[5] by invoking the Curtin-Hammett principle, according to which the reaction pathway for a molecule that exists as an equilibrium mixture of two different conformers is independent of their relative abundance if conformational interconversion is rapid in comparison with subsequent chemical reactions. Thus, a minor conformer ($^4C_1$) could determine the course of periodate oxidation, with the major conformer ($^1C_4$) acting solely as an unreactive reservoir.

A second aspect of the controversy is that X-ray fiber diffraction evidence[6] is incompatible with standard $^1C_4$ geometry for the L-iduronate ring and instead favors the alternative $^4C_1$ conformation. In view of the small energy difference between the two chair forms, conformational rearrangement in response to packing constraints in the solid state is entirely feasible. However, direct investigation requires techniques applicable to both the solid and solution states. Chiroptical methods allow such comparison.

In the present work we have reinvestigated the conformation of the iduronate ring in dermatan sulfate solutions, using NMR at 500 MHz to confirm the conclusions from the original study at 270 MHz, and have made a direct comparison of the circular dichroism (CD) of dermatan sulfate in solutions and in solid films. CD originates from temporal correlations between the electric

[†] State University of New York.
[‡] Silsoe College.
[§] National Institute for Medical Research.

(1) Arnott, S.; Rees, D. A.; Morris, E. R. In *Molecular Biophysics of the Extracellular Matrix*: Humana; Clifton, N.J., **1984**.
(2) Stoddart, J. F. *Stereochemistry of Carbohydrates*; Wiley: New York, **1971**.
(3) Gatti, G.; Casu, B.; Torri, G.; Vercellotti, J. R. *Carbohydr. Res.* **1979**, *68*, C3-C7.
(4) Fransson, L.-A. *Carbohydr. Res.* **1974**, *36*, 339-348.
(5) Rees, D. A.; Morris, E. R.; Stoddart, J. F.; Stevens, E. S. *Nature (London)* **1985**, *317*, 480.
(6) Mitra, A. K.; Arnott, S.; Atkins, E. D. T.; Isaac, D. H. *J. Mol. Biol.* **1983**, *169*, 873-901.

0002-7863/86/1508-3790$01.50/0

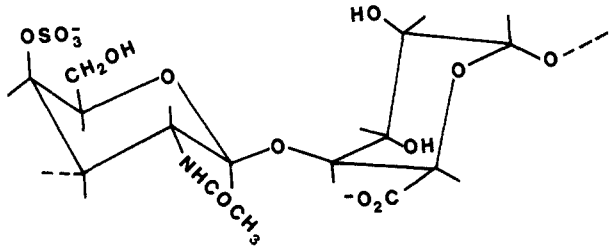

Figure 1. Dominant repeating disaccharide unit in dermatan sulfate with α-L-IdA residues shown in the $^1C_4$ ring conformation.

dipole and magnetic dipole transition moments of a molecule in the absence of applied fields and is therefore very sensitive to the overall spatial arrangement of constituent atoms and bonds. Applied to glycosaminoglycans, it has been used as a probe of molecular conformation in solution and of changes in conformation elicited by environmental perturbation (e.g., pH, temperature, ionic strengh).[7]

Extension of CD measurements to the vacuum ultraviolet region reveals optically active transitions originating from the constituent rings and interresidue linkages of the polymer chain, rather than only from substituent groups. Vacuum ultraviolet circular dichroism (VUCD) spectra of chondroitins have been published previously.[8] We now report an investigation of the spectroscopic and conformational changes resulting from C(5) epimerization of β-D-GlcA, as in chondroitin, to α-L-IdA in dermatan.

Experimental Section

Dermatan sulfate, from porcine skin, was obtained as the sodium salt from Sigma Chemical Co. Amino acid analysis showed that the protein content of the sample was less than 0.8%. Carbon analysis (26.05%) indicated that the sample contained 22.0% water; sulfur analysis indicated that sulfation was essentially complete, giving a molecular weight, per disaccharide, of 503. The ^{1}H NMR spectrum (see below) contains weak resonances reflecting the presence of glucuronate residues,[3] from which we estimate the glucuronate content to be no greater than 10% of the iduronate, similar to what was observed in the earlier NMR study.[3] These values were used in calculating molar ellipticities.

Solutions were prepared by dissolving the sample directly in D_2O. VUCD measurements were made on a prototype instrument described previously.[9] Spectra were obtained with concentrations of 8.7, 4.0, and 1.5 mg/mL in path lengths of 0.5 or 0.1 mm. Scan rates were 1.0 or 2.0 nm/min with time constants of 100 or 30 s; spectral resolution was 3.2 nm. Under these conditions aqueous solutions could be measured to 170 nm. Films were cast on calcium fluoride disks by evaporating solutions to dryness in a desiccator. To detect any molecular orientation, successive spectra were obtained as the film was rotated in the light beam by increments of 90°.

In collaboration with Dr. J. C. Sutherland spectra to 170 nm were also obtained at the U.S. National Synchrotron Light Source at Brookhaven National Laboratory. The spectrometer parameters were approximately the same as those described above, the significant difference being the use of the high-intensity synchrotron light source in place of a conventional hydrogen-discharge lamp.

Solution spectra were also obtained to 185 nm on a Jobin Yvon Auto Dichrograph Mark V circular dichroism spectrometer. Solutions of 0.125 mg/mL concentration were measured in a 2.0-mm path length cell with a 2.0-nm band pass and 5-s time constant. These results were used to confirm the ellipticity values obtained at a shorter path length on the prototype spectrometers.

Proton NMR spectra were obtained on a Brucker 500-MHz spectrometer. Solutions of 12 mg/mL concentration were deuterium exchanged by repeated treatment with D_2O. A total of 2500 scans were taken at 343 K, and standard resolution enhancement was applied. Chemical shifts and assignments were previously reported by Gatti et al.,[3] and the main purpose of our examining the proton NMR was to confirm the ring conformation of the α-L-IdA moiety in our solution sample by examination of spin–spin coupling constants.

Results

The spectrum of dermatan sulfate in D_2O, pD 7.0, shown in Figure 2, is that obtained at the U.S. National Synchrotron Light Source at Brookhaven National Laboratory. It is similar in all respects to the spectrum obtained on the prototype VUCD instrument described earlier,[9] except that the signal-to-noise ratio is significantly improved at 175 nm, reflecting the greater intensity of the synchrotron light source relative to a conventional hydrogen-discharge lamp.

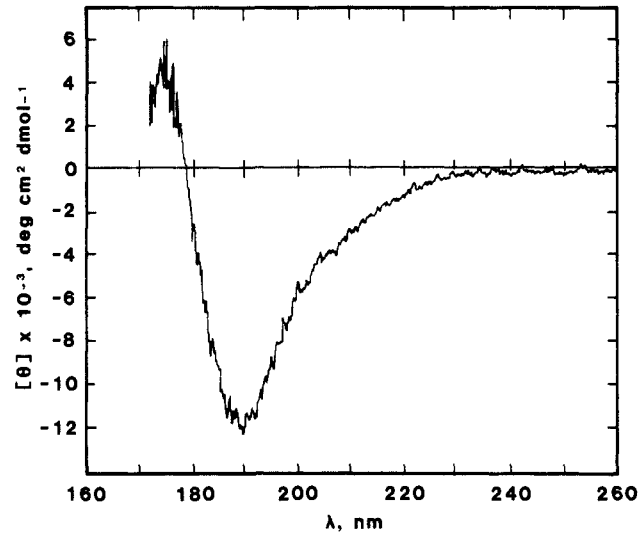

Figure 2. Circular dichroism of dermatan sulfate, aqueous solution. Molar ellipticity, [θ], is given per disaccharide.

A negative CD band appears at 189 nm with molar ellipticity $[\theta] = -11.9 \times 10^3$ deg cm^2 dmol^{-1}. The shape of the long-wavelength CD indicates the presence of a second negative band; the molar ellipticity near 212 nm is approximately -2.4×10^3 deg cm^2 dmol^{-1}. A positive CD band is located near 175 nm with molar ellipticity $[\theta] = +4.8 \times 10^3$ deg cm^2 dmol^{-1}.

Film spectra, measured to 155 nm, were qualitatively the same as the solution spectra in the overlapping region. There was an orientation dependence in the intensities of the CD bands, indicating a partial ordering of polymer chains, film thickness inhomogeneities, or both. There was evidence of "ridging" whereby the outer rim of the film appeared thicker than the center through which the light beam passes. The amount of material actually sampled by the light beam can be estimated by comparing the UV absorption of the film with that of a solution of known concentration and assuming equal absorption coefficients in solution and film. Doing so for the film spectrum averaged over the several orientations used in the experiment indicates that the molar ellipticities of the negative and positive CD bands are approximately the same in the film as in solution, although the uncertainty is large (20–25%). Film spectra indicate a slight red shift of the negative band to 192 nm. The crossover is at 178 nm, and the positive band appears at approximately 170 nm. Below 170 nm the CD of films decreases in intensity, approaching zero near the cutoff of the measurements at 155 nm. Of special importance for our purposes is the observation that the ratio of the intensity of the large negative band to that of the short-wavelength positive band is approximately 2.5 in both film and solution CD spectra.

Our 500-MHz proton NMR spectrum of dermatan sulfate at 343 K showed the same chemical shifts and spin–spin coupling constants as the previously published spectrum obtained at 270 MHz.[3] It is the small coupling constants of the three low-field proton resonances, which were used earlier to demonstrate the $^1C_4$ ring conformation of the α-L-IdA moiety. Specifically, the $^4C_1$ conformation is precluded by the narrow I(1) resonance, since the trans-diaxial H(1) and H(2) protons of the $^4C_1$ conformation would give a characteristically large coupling constant, >7 Hz.[3]

Discussion

The CD of dermatan sulfate above 185 nm was previously reported by Stone[10,11] and by Park and Chakrabarti.[12] Stone[10,11]

(7) Morris, E. R.; Frangou, S. A. In *Techniques in Carbohydrate Metabolism*; Elsevier: London, **1981**; Vol. B308, pp 109–160.
(8) Stipanovic, A. J.; Stevens, E. S. *Biopolymers* **1981**, *20*, 1565–1573.
(9) Pysh(Stevens), E. S. *Annu. Rev. Biophys. Bioeng.* **1976**, *5*, 63–75.
(10) Stone, A. L. *Biopolymers* **1969**, *7*, 173–188.

observed a negative band at 188 nm with a molar ellipticity of $[\theta] = -16400$ deg cm^2 dmol^{-1} and a weaker negative band at 212 nm with $[\theta] = -4500$ deg cm^2 dmol^{-1}. Park and Chakrabarti[12] observed a shoulder in the negative dichroism near 212 nm, rather than a discrete negative band.[10,11] At 212 nm they found a molar ellipticity of $[\theta] = -4800$ deg cm^2 dmol^{-1}. Park and Chakrabarti did not reach the extremum of the negative band just below 190 nm, but their spectrum shows a molar ellipticity at 190 nm of $[\theta] = -12000$ deg cm^2 dmol^{-1}, consistent with Stone's data. Our results are in agreement with the previous work, given the expected variation in molar ellipticities arising from sample heterogeneity, e.g., differing amounts of β-D-GlcA present as a minor component of uronic acid and contamination with other glycosaminoglycans. Molar ellipticities are calculated from observed dichroism on the basis of sample purity, water content, and extent of sulfation. Stone used amino sugar analysis as a basis for the calculation of molar ellipticities.[10,11] Park and Chakrabarti used hexuronic acid analysis.[12] We used carbon analysis to determine water content and sulfur analysis to determine extent of sulfation. One feature the reported spectra have in common is the ratio of molar ellipticity at 189 nm to that at 212 nm, the ratio being approximately equal to 4 in all reported spectra.

n–π* Transitions. The negative dichroism in glycosaminoglycans near 212 nm has been assigned to the n–π* transitions of the acetamido and carboxyl groups, with the acetamido contribution usually being the larger, but with the carboxyl group determining the CD changes that occur during changes in pH and cation binding.[10,13] Morris et al.[14] have described the CD properties of uronic acids above 190 nm and, in particular, have shown that C(5) epimerization, i.e., β-D-GlcA vs. α-L-IdA, significantly alters the near-UV CD.

Coduti et al.[15] have shown that the CD of the n–π* transition in 2-acetamido-2-deoxy sugars is not very sensitive to anomeric configuration. Compounds having the D-gluco or D-galacto configuration display a negative CD band centered in the range 209–212 nm, with a molar ellipticity between −3000 and −5000 deg cm^2 dmol^{-1}. The CD is also not strongly modified by methylation at O(3), mimicking the formation of a disaccharide linkage. In a series of chitin oligosaccharides the negative ellipticities increased in magnitude from −3000 to −7000 deg cm^2 dmol^{-1} on going from chitobiose to chitohexaose.[15] At 212 nm the molar ellipticity of methyl-2-acetamido-2-deoxy-β-D-galactopyranoside, modeling the amino sugar of dermatan sulfate, is −3700 deg cm^2 dmol^{-1} (Figure 3).

In their study of the n–π* band in uronic acids, Morris et al.[14] reported a simple negative 210-nm band for the sodium salt of methyl-α-L-iduronoside, with a molar ellipticity of $[\theta] = -5430$ deg cm^2 dmol^{-1} (Figure 3).

Therefore, if the CD of dermatan sulfate near 210 nm were the simple sum of contributions from noninteracting amino sugar and uronic acid moieties, one would expect a disaccharide molar ellipticity approximately twice that actually observed (Figure 3). There is a marked decrease in the polymer CD relative to the sum of monomer spectra, the difference being greater than the experimental uncertainties in measured molar ellipticities.

On the other hand, there is apparent simple additivity of monomeric n–π* CD in the case of chondroitin, chondroitin 4-sulfate, and chondroitin 6-sulfate, all of which have molar ellipticities near 210 nm in the range -5.9×10^3 to -6.8×10^3 deg cm^2 dmol^{-1}.[7,12,16-18] (Eyring and Yang[16] reported molar ellipticities

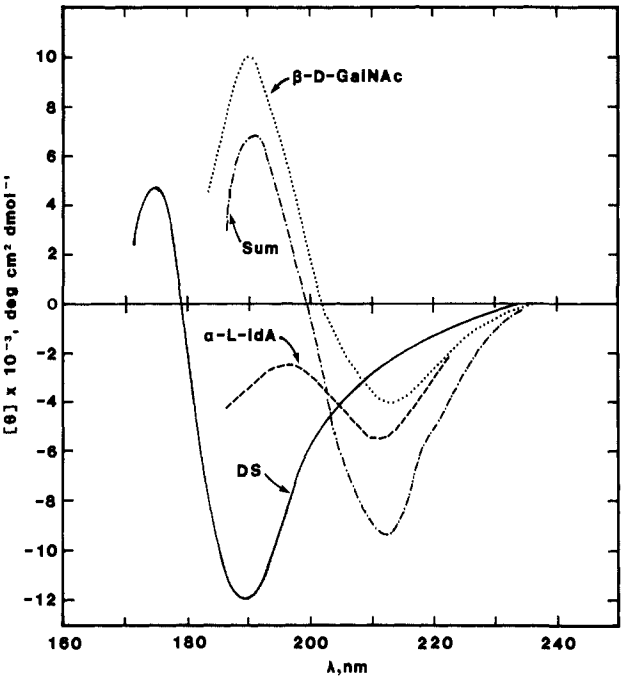

Figure 3. Comparison of dermatan sulfate CD (—), the sum of the CD of monomeric model compounds (···), and the CD of each of the monomeric model compounds methyl-β-D-galactopyranoside (···, and methyl-α-L-iduronoside (- - -).

based on an averaged monomer molecular weight that must be doubled for comparison with ellipticities per disaccharide unit. Values reported in ref 17 are corrected in ref 18.) These values are only slightly larger than the sum of molar ellipticities of methyl-2-acetamido-2-deoxy-β-D-galactopyranoside and methyl-β-D-glucuronoside, the constituents of chondroitins.

Chondroitin and dermatan have the same amino sugar moiety, with axial O(4)H. Indeed, the only structural difference is epimerization at C(5) of the uronic acid moiety, giving rise to a change from the $^4C_1$ ring conformation in chondroitin to the $^1C_4$ ring conformation in solutions of dermatan. That difference results in very different n–π* rotational strengths in the monomers, with β-D-glucuronate, present in chondroitin, having a much smaller n–π* CD band than α-L-iduronate, present in dermatan. But since chondroitin itself has a larger n–π* CD band than dermatan, the epimerization must also be responsible for the large nonadditivity observed in dermatan but not in chondroitin. Precisely which interactions are responsible is not yet known, but a leading possibility is that the (1→3) linkage in dermatan, being axial–equatorial in the $^1C_4$ form of iduronate, has a different dihedral angle at C(3)–O(3) than in chondroitin, in which the (1→3) linkage is equatorial–equatorial.

π–π* Transitions. The CD of glycosaminoglycans near 190 nm has been assigned to the π–π* transition of the acetamido group.[11] In dermatan sulfate (Figure 2) the CD at 189 nm is strongly negative, whereas the summed CD of the monomers, methyl-2-acetamido-2-deoxy-β-D-galactopyranoside and methyl-α-L-iduronoside, is positive (Figure 3). The origin of this nonadditivity has not been determined, but the magnitude of the nonadditivity indicates that the disaccharide interactions are substantial.

The π–π* transition of the carboxyl group observed in simple uronic acids in the region 185–190 nm[14] is likely to contribute to the 189-nm band in dermatan sulfate as well. It is probably not a major source of rotational strength of the 175-nm band (see below), that wavelength being too low for such an assignment.

In an analysis of the 188–190-nm CD of hyaluronic acid, Cowman et al.[18] showed that, although the observed CD appears to be close to the sum of the CD of noninteracting monomeric constituents, there is, in fact, a large negative CD contribution from the (1→3) uronic acid–amino sugar linkage and a large positive CD contribution from the (1→4) amino sugar–uronic acid

(11) Stone, A. L. *Biopolymers* **1971**, *10*, 739–751.
(12) Park, J. W.; Chakrabarti, B. *Biochim. Biophys. Acta* **1978**, *544*, 667–675.
(13) Beychok, S.; Kabat, E. A. *Biochemistry* **1965**, *4*, 2565–2574.
(14) Morris, E. R.; Rees, D. A.; Sanderson, G. R.; Thom, D. *J. Chem. Soc. Perkin Trans. 2* **1975**, 1418–1425.
(15) Coduti, P. L.; Gordon, E. C.; Bush, C. A. *Anal. Biochem.* **1977**, *78*, 9–20.
(16) Eyring, E. J.; Yang, J. T. *Biopolymers* **1968**, *6*, 691–701.
(17) Cowman, M. K.; Balazs, E. A.; Bergmann, C. W.; Meyer, K. *Biochemistry* **1981**, *20*, 1379–1385.
(18) Cowman, M. K.; Bush, C. A.; Balazs, E. A. *Biopolymers* **1983**, *22*, 1319–1334.

linkage that cancel each other almost completely in the polymer CD spectrum.

Stone[11] had early on noted a correlation between the sign of the 190-nm CD of glycosaminoglycans and the linkage site, such that (1→4) linked amino sugars display large positive CD near 190 nm, whereas those with (1→3) linkage (e.g., dermatans, hyaluronic acid, chondroitins) have small or negative CD in the π–π^* amide transition region. Coduti et al.[15] offered some substantiating evidence for the validity of this generalization in noting, for example, that methylation at O(3) in 2-acetamido-2-deoxyglucopyranoside changes the positive 190-nm CD to a small negative value.

The results of Cowman et al.[18] on hyaluronic acid are consistent with this generalization insofar as the (1→3) uronic acid–amino sugar linkage was found to be a source of large negative CD.

Vacuum Ultraviolet CD. Since the first report[19] of vacuum ultraviolet CD measurements for a polysaccharide (iota carrageenan) in 1975, the data bank has expanded rapidly, and VUCD spectra have now been obtained for a wide variety of polysaccharides, including glucans,[20–25] algal polysaccharides,[19,26,27] galactomannans,[28] chitins,[29] galacturonates,[30] and glycosaminoglycans.[8,18,31] In the absence of water, spectra can, in favorable cases, be recorded to ~140 nm. A common feature of such solid polysaccharide films is the presence of two VUCD bands of opposite sign, separated in wavelength by ~20 nm. The higher energy (short-wavelength) band is invariably the more intense and generally dominates the sign of optical activity at long wavelength (e.g., the sodium D line). The wavelength of the weaker, lower energy transition is usually close to that of the positive VUCD band reported here for dermatan sulfate (~175 nm).

Spectra of glycopyranoses and glycopyranosides[32,33] provide evidence for an optically active transition near 175 nm associated with the unsubstituted sugar ring. Since electronic excitation of the C–C and C–H groups would be expected at an even shorter wavelength, workers have generally agreed that sugar-ring transitions in the 175-nm region reflect excitation of the oxygen-containing chromophores, with the excited electron originating in the nonbonding n orbital of the oxygen atom. The ring oxygen, O(5), and glycosidic oxygen, O(1), considered either separately or together as an acetal or hemiacetal chromophore, have been the usual focus of attention in assigning the lowest energy transitions of the sugar ring.

Although it is recognized that hydroxyl transitions should have similar excitation energies, the rotational freedom of these groups will in general argue against any substantial contribution to net dichroism, although this point has not been definitely resolved. The partial correlation of the sign of the 175-nm CD band with anomeric configuration in glycopyranosides[33] implicates the linkage oxygen, O(1), as one important determinant of the CD, but its role could be either as the chromophore itself or as a dominant perturber of the actual chromophore.

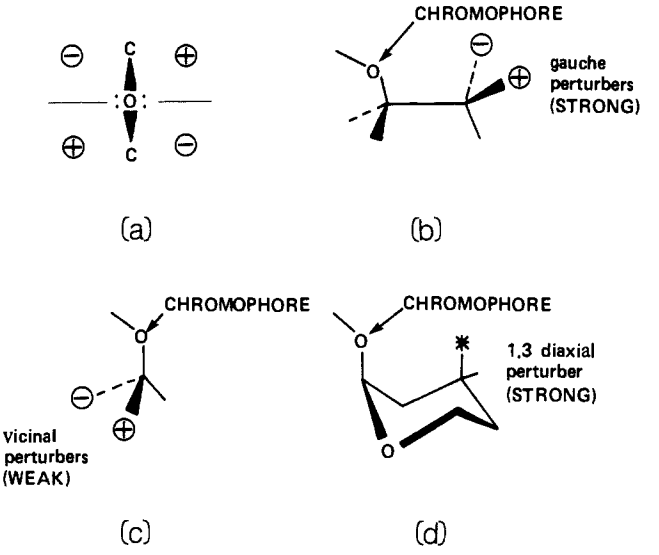

Figure 4. Perturbation of acetal oxygen chromophores [O(1) and O(5) in hexapyranose residues] by oxygen-containing groups close to the chromophore: (a) quadrant rule proposed to rationalize the sign of the VUCD band at ~175 nm, attributed to excitation of the nonbonding oxygen electrons (The molecule is viewed with the chromophoric oxygen atom projecting toward the observer, as indicated); (b) strong perturbation by oxygen-containing groups lying close to the nonbonding electrons, in positions gauche to the chromophore across a carbon–carbon bond; (c) weak perturbation by substituents bonded directly to the same carbon atom as the chromophore, but oriented away from the nonbonding electrons; (d) strong perturbation of O(1) axial by an axial substituent at C(3).

In Figures 4 and 5 we outline a tentative proposal for rationalization of the sign of polysaccharide VUCD at ~175 nm, in terms of a simple spatial rule. This proposal offers a unified interpretation of published VUCD results for other polysaccharides and provides a basis for discussion of the positive band at 175 nm in the VUCD of dermatan sulfate.

The starting point in application of this proposed treatment is to regard the two oxygen constituents of the acetal group as separate chromophores, both contributing to the observed CD at ~175 nm, and to divide the space around each into four quadrants defined by the symmetry planes of the chromophore (Figure 4a). Oxygen-containing groups (OH; CH_2OH; COO^-, $NH{\cdot}CO{\cdot}CH_3$, etc.) close to the chromophore are then regarded as "perturbers", inducing either positive or negative CD activity according to the quadrant in which they lie.

Groups lying close to the nonbonding electrons of the chromophore are regarded as "strong" perturbers. For the constituent sugars of all polysaccharides so far studied by VUCD, only two geometric arrangements give rise to a close interaction of this type: (1) when the chromophore and perturber occupy gauche positions relative to one another across a C–C bond (Figure 4b) and (2) when O(1) is axial and the oxygen-containing substituent at C(3) is also axial (Figure 4d). Groups lying close to the chromophore but "behind" it (i.e, away from the nonbonding electrons) are regarded as "weak" perturbers (Figure 4c). The most common interaction of this type would be between O(5) and O(1) axial. Oxygen-containing substituents lying on one of the symmetry planes of the chromophore [e.g., perturbation of O(5) by O(1) equatorial] will make no net contribution to induced CD.

In prediction or rationalization of the sign of the CD band we first consider strong perturbations; if these are absent or if oppositely signed contributions cancel out, weak perturbations are then taken into account. At this level of approximation we regard different oxygen-containing perturbers as equivalent, although clearly a more rigorous treatment would be required for quantitative interpretation of CD intensity.

(19) Balcerski, J. S.; Pysh (Stevens), E. S.; Chi-Chen, G.; Yang, J. T. *J. Am. Chem. Soc.* **1975**, *97*, 6274–6275.
(20) Lewis, D. G.; Johnson, W. C., Jr. *Biopolymers* **1978**, *17*, 1439–1449.
(21) Stipanovic, A. J.; Stevens, E. S.; Gekko, K. *Macromolecules* **1980**, *13*, 1471–1473.
(22) Stipanovic, A. J.; Stevens, E. S. *Int. J. Biol. Macromol.* **1980**, *2*, 209–212.
(23) Stipanovic, A. J.; Stevens, E. S. In *ACS Symposium Series, No. 150*; Brant, D. A., Ed.; American Chemical Society: Washington, D.C. **1981**; pp 303–315.
(24) Stipanovic, A. J.; Stevens, E. S. *Biopolymers* **1981**, *20*, 1183–1189.
(25) Stipanovic, A. J.; Stevens, E. S. *J. Appl. Polymn. Sci.* **1983**, *37*, 277–281.
(26) Liang, J. N.; Stevens, E. S.; Morris, E. R.; Rees, D. A. *Biopolymers* **1979**, *18*, 327–333.
(27) Liang, J. N.; Stevens, E. S.; Frangou, S. A.; Morris, E. R.; Rees, D. A. *Int. J. Biol. Macromol.* **1980**, *2*, 204–208.
(28) Buffington, L. A.; Stevens, E. S.; Morris, E. R.; Rees, D. A. *Int. J. Biol. Macromol.* **1980**, *2*, 199–203.
(29) Buffington, L. A.; Stevens, E. S. *J. Am. Chem. Soc.* **1979**, *101*, 5159–5162.
(30) Liang, J. N.; Stevens, E. S. *Int. J. Biol. Macromol.* **1982**, *4*, 316–317.
(31) Buffington, L. A.; Pysh (Stevens), E. S.; Chakrabarti, B.; Balazs, E. A. *J. Am. Chem. Soc.* **1977**, *99*, 1730–1734.
(32) Nelson, R. G.; Johnson, W. C., Jr. *J. Am. Chem. Soc.* **1976**, *98*, 4290–4295.
(33) Nelson, R. G.; Johnson, W. C., Jr. *J. Am. Chem. Soc.* **1976**, *98*, 4296–4301.

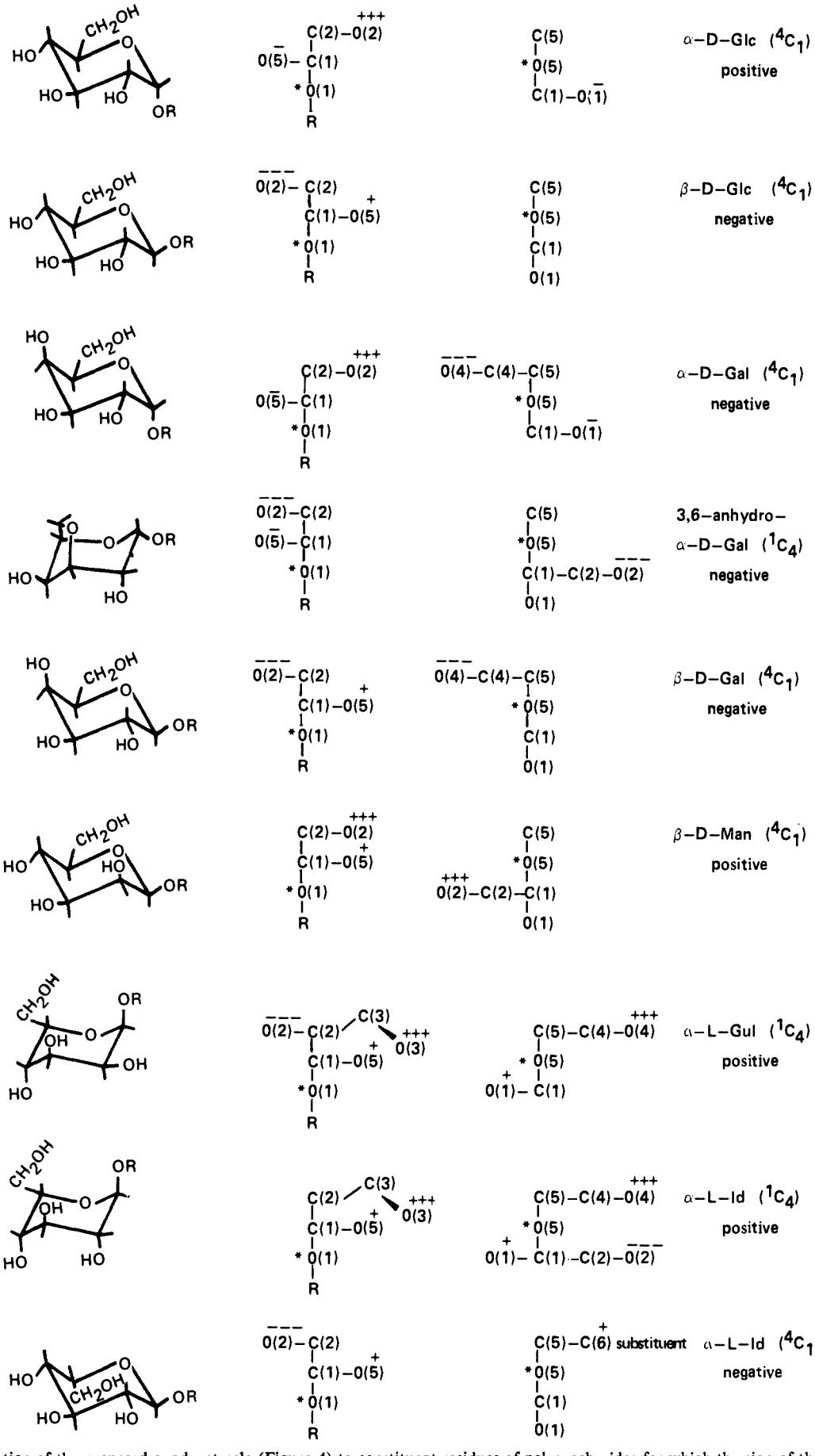

Figure 5. Application of the proposed quadrant rule (Figure 4) to constituent residues of polysaccharides for which the sign of the VUCD band at ~175 nm is known. In each projection the chromophoric oxygen is identified by an asterisk, strong perturbation is denoted by a triple sign (+++ or − − −) the appropriate group, and weak perturbation is denoted by a single sing (+ or −). The predicted sign of the resulting net CD at ~175 nm is shown on the right of the figure, under the residue name.

The disposition of other groups relative to the O(5) chromophore is fixed by the stereochemistry of the sugar ring. The spatial arrangement of adjacent residues about the glycosidic oxygen, O(1), however, is the principal determinant of polysaccharide chain geometry. For application of the proposed sector rule, we have used (Figure 5) a projection based on the preferred conformation of sugar glycosides, in which C(2) lies in the C(1)–O(1)–CH_3 plane. The known sensitivity of polysaccharide optical activity to chain conformation, including the change in intensity of the VUCD band at ~175 nm on adoption of the ordered conformation of agarose,[26] demonstrates that this treatment can be regarded only as a first approximation. For most polysaccharides, however, steric restriction across the glycosidic linkage limits conformational mobility to a comparatively narrow range of dihedral angle, so it is unlikely that departure from the standardized "preferred" orientation of O(1) will affect the sign (as opposed to the magnitude) of the CD band.

Figure 5 shows the application of the proposed "sector rule" to the constituent sugars of the polysaccharides for which VUCD spectra are currently available. The agreement with experimental results is excellent, as detailed below for specific systems.

(1) The only "strong" interaction for α-D-glucose is between O(1) and O(2), giving rise to a positive CD prediction. This is consistent with the positive CD band observed at ~175 nm in all the α-D-glucans so far studied (amylose, amylopectin, glycogen, dextran, nigeran, pseudonigeran, pullulan),[20,23] irrespective of whether the interresidue linkage is (1→3), (1→4), (1→6), or some combination of these.

(2) In β-D-glucose the only strong interaction is again between O(1) and O(2), but the change in anomeric configuration leads to a change of predicted sign from positive to negative. This is consistent with the observed negative band at ~175 nm in the VUCD of curdlan [(1→3)-β-D-glucan].[23] Cellulose [(1→4)-β-D-glucan] is anomalous in showing a single, very intense, negative VUCD at ~155 nm.[23] A possible interpretation of this behavior is that the extensive hydrogen-bonding characteristic of the cellulose II crystal structure greatly enhances the optical activity of the hydroxyl groups of the sugar ring by locking them in a specific steric arrangement. However, films of chitin [which is based on a cellulose backbone, but with the hydroxyl group at C(2) replaced by an *N*-acetyl substituent] show a typical "positive-negative doublet" below 180 nm, with the longer wavelength lobe having the predicted negative sign.[29]

(3) Films of polygalacturonate [a (1→4)-linked polymer of α-D-galacturonate] in both the free acid and the sodium salt form also show a positive–negative doublet below 180 nm.[30] The negative sign of the longer wavelength (~172 nm) component is in agreement with the predicted sign (Figure 5) for α-D-galactose (and its uronic acid derivative).

(4) The predicted sign is also negative for β-D-galactose and for 3,6-anhydro-α-D-galactose, consistent with the observed negative sign of the band at ~179 nm in the VUCD spectrum of iota carrageenan[19] (a sulfated algal polysaccharide based on an alternating repeating sequence of 3-linked β-D-galactose and 4-linked 3,6-anhydro-α-D-galactose). The corresponding VUCD band is positive for agarose,[26] a structurally related polysaccharide in which 3,6-anhydro-α-D-galactose is replaced by the mirror-image residue 3,6-anhydro-α-L-galactose, with consequent inversion of the predicted sign of optical activity.

(5) Analysis of the VUCD spectra of galactomannans[28] of varying galactose content shows a positive contribution to the band at ~170 nm from the (1→4)-linked β-D-mannan backbone and a negative contribution from the α-D-galactose side chains attached at C(6) of some of the backbone residues. This is consistent with the positive sign predicted (Figure 5) for β-D-mannose and the negative sign predicted for α-D-galactose.

(6) Alginate, a (1→4)-linked copolymer of β-D-mannuronate and α-L-guluronate, shows a positive VUCD band at ~170 nm,[27] in agreement with the positive sign predicted for both β-D-mannose and α-L-gulose (and their uronic acid derivatives).

(7) In the chondroitins (which are based on an alternating repeating sequence of 3-linked β-D-galactosamine and 4-linked β-D-glucuronate) the CD at 175 nm is negative.[8] This is consistent with the negative sign predicted (Figure 5) for β-D-galactose and β-D-glucose (and their 2-acetamido-2-deoxy and uronic acid derivatives).

The success of the proposed "quadrant rule" in rationalizing the sign of the VUCD band at ~175 nm for all polysaccharides where this has been unequivocally determined lends confidence to its application to dermatan sulfate. The predicted sign (Figure 5) for β-D-galactose, and hence for the 3-linked β-D-galactosamine residues of dermatan sulfate (Fig. 1), is negative. The prediction for α-L-iduronate in the $^4C_1$ chair conformation identified by X-ray diffraction analysis of oriented dermatan sulfate fibers is also negative, in contrast to the positive VUCD band at ~175 nm (Figure 2) observed both in solution and in solid films. Inversion of the iduronate ring conformation to the normal $^1C_4$ chair form [C(6) equatorial], however, changes the predicted sign to strongly positive. Our results are therefore consistent with NMR evidence of $^1C_4$ ring geometry in solution and further indicate that there is no substantial change in molecular organization on drying down to the solid state, in apparent conflict with the conclusions from fiber diffraction.

One possible explanation is that, in the condensed phase, dermatan sulfate exists predominantly in an amorphous form with the same iduronate ring geometry as in solution but that X-ray diffraction patterns are dominated by a minor polycrystalline component in which the iduronate ring adopts the alternative $^4C_1$ chair form, perhaps to facilitate packing with the D-glucuronate residues that occasionally replace L-iduronate in the polymer chain, and whose ring geometry is effectively locked in the $^4C_1$ conformation.

An alternative possibility (Arnott, S., personal communication) is that a distorted $^1C_4$ L-iduronate ring conformation, not considered in the initial X-ray analysis, may provide a single, unifying interpretation of both the solution NMR and fiber diffraction evidence.

Acknowledgment. This work was supported by NIH Grant GM24862 and NATO Grant 705/83. Some VUCD spectra were obtained in collaboration with Dr. J. C. Sutherland at the U.S. National Synchrotron Light Source at Brookhaven National Laboratory. We are also grateful to Dr. J. Feeney for the 500-MHz NMR spectrum obtained at the National Institute for Medical Research, Mill Hill.

Registry No. Dermatan sulfate, 24967-94-0.