Conformational Properties of β-(1→4)-D-Galactan Determined from Chiroptical Measurements

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Received March 9, 1990; Revised Manuscript Received June 19, 1990

ABSTRACT: A chiroptical method of conformational analysis is applied to the neutral pectic polysaccharide β-(1→4)-D-galactan. The method is based on experimental measurements of Na_D optical rotation and vacuum-UV CD and a recently developed calculational model for saccharide optical activity. We conclude that the solution conformation is well described by the linkage geometry $(\phi,\psi) = (60°,40°)$, in agreement with calculated potential energy contours; fluctuations about this conformation are relatively limited. The solid-state conformation is determined to within a specific range of (ϕ,ψ) values, including a conformation only slightly different from the solution conformation. A second possibility for the solid-state conformation is $(\phi,\psi) = (160°,20°)$, also not energetically unfavorable.

Introduction

Chiroptical properties, such as optical rotation and circular dichroism (CD), are known for their sensitivity to variations in molecular structure and conformation. That sensitivity, however, makes it difficult to extract absolute structural information from experimental data; any interpretive model must relate not only correctly but in rather detailed fashion the dependence of chiroptical properties on the geometric arrangement of atoms. Recent advances in the experimental measurement and the interpretation of saccharide chiroptical properties have provided a new method of disaccharide conformational analysis.[1] We have here extended that method to a conformational analysis of the neutral pectic polysaccharide β-(1→4)-D-galactan.

The chiroptical analysis includes a comparison of the experimentally measured Na_D optical rotation with the rotation calculated as a function of the linkage dihedral angles ϕ and ψ; the comparison displays which linkage geometries are compatible with the observed rotation.[1] A comparison can also be made of the measured vacuum-UV CD (VUCD) and the VUCD calculated as a function of linkage geometry. The method is intended to complement fiber X-ray diffraction[2,3] and NMR techniques.[4,5] NMR has been useful in the conformational analysis of small oligosaccharides in solution but is often limited in its application to polysaccharides on account of line broadening.[6] The chiroptical analysis can usefully be correlated, as will be shown, with molecular modeling methods that employ one or another empirical potential energy function to describe conformational preferences[4,5,7-10] and provides an independent means of evaluating potential energy functions. In applications to maltose and cellobiose,[1] the conclusions that were drawn were found to be similar to some, but not all, earlier analyses based on energy calculations and NMR.

Although β-(1→4)-D-galactans devoid of other sugar residues are rare, they are found in arabinogalactans with up to 25% L-arabinofuranose residues in side chains, in compression wood in combination with 5–10% galacturonate or glucuronate residues, and in pectic side chains.[11-14] No fiber diffraction or NMR analyses have been reported for β-(1→4)-D-galactan, but energy calculations[7,15,16] indicate that, relative to amylose and cellulose, β-(1→4)-D-galactan is intermediate with respect to chain stiffness and extension. In helix type it resembles amylose because its glycosidic and aglycon bonds include both equatorial and axial types, resulting in similar "ring twists".[15] The β-configuration at C(1), however, makes the glycosidic bond equatorial, and the range of ϕ-values free of steric contacts resembles cellulose more than amylose.[7,15,16]

The relationship between the structure of a polysaccharide and its physical and biological properties is partially determined by the conformational preferences of the polymer. The method we describe here is general and can be applied to a broad range of polysaccharide structures.

Calculational Methods

The method we use to calculate the VUCD and Na_D molar rotation, $[M]$, has previously been described in detail,[17] and its applicability to pyranoses,[18] pyranosides,[19] other model compounds,[19,20] and disaccharides[1] demonstrated. Its main feature is the explicit reference to the high-energy optically active σ–σ^* electronic transitions deep in the vacuum UV which determine optical rotation and CD. It is based on the Kirkwood theory of optical activity[21,22] in which the high-energy electric-dipole-allowed transitions localized on an individual bond are summed to express the bond polarizability; the molecular optical rotation in the visible region of the spectrum is then described as the result of the interaction among bond polarizabilities. In our model the bond-localized electric-dipole-allowed transitions are summed to express a single transition moment localized on each of the bonds. Perturbation theory is then used to describe their interaction, resulting in molecular transition moments, each of which is expressed as a linear combination of bond moments. The molecular transition moments, through well-known equations,[23,24] determine the CD and, via a Kronig–Kramers transform, the optical rotation. The advantage of this procedure is that it yields not only the molecular optical rotation in the visible region of the spectrum but also the VUCD, which has recently become experimentally accessible, thereby allowing two means of comparison with experiment.

In the present work we used the same parameterization as was originally optimized for saccharide fragments (e.g.,

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CH_2OH–CH_2OH).[17] Each galactose residue in the chain was taken to be in the $^4C_1$(D) ring conformation with atomic coordinates adapted from the glucose coordinates of Arnott and Scott.[24] Hydroxyl hydrogen atoms do not enter into the calculation; their coordinates need not be specified.

Within the glycosidic linkage the C–O–C bond angle was taken to be 117.5°.[25-27] The linkage conformation is then specified by the angles ϕ and ψ, where ϕ = 0° corresponds to the C(1)–H bond cis to the O(1)–C′(4) bond, ψ = 0° corresponds to the O(1)–C(1) bond cis to the C′(4)–H bond, and positive values of ϕ and ψ refer to clockwise rotation of the reducing residue as viewed from the nonreducing residue. We considered only regular chain conformations, i.e., chain segments in which all linkage conformations are the same (see Discussion). The linkage conformations we examined were those within the range of (ϕ,ψ) values found by Brant to be free of extreme steric contacts.[7]

The calculations refer to molecules in a vacuum. For comparison with aqueous solution data, a solvent correction $(n^2+2)/3 = 1.26$ can be applied, where n is the solvent refractive index.[28] We also apply an empirical scale factor of 1.69 to the calculated results, which brings monomer calculations into almost quantitative agreement with experimental data[1] and which may be related to optical activity contributions omitted in the Kirkwood theory.[1]

Hydroxymethyl groups were placed either all in the gt or tg conformation. (The notation specifies the orientation of the C(6)–O(6) bond, gauche or trans, relative to the C(5)–O(5) bond (first letter) and to the C(5)–C(4) bond (second letter).) Following Lemieux and Brewer,[29] we took the gt and tg conformations to be equally probable such that, for a galactose monomer, the weighted average rotation is given by

$$[\bar{M}]^{\text{calcd}} = 0.50[M]^{\text{calcd}}_{\text{gt}} + 0.50[M]^{\text{calcd}}_{\text{tg}}$$

If these stastical weights are retained in oligomers, the weighted average we require for a trimer (for example) is given by

$$[\bar{M}]^{\text{calcd}} = 0.125\sum[M]^{\text{calcd}}_{\alpha,\beta,\gamma}$$

where $0.125 = 0.50^3$ and α,β,γ = gt or tg. We assume that the contribution of each hydroxymethyl group in an oligomer to optical rotation is independent of the conformation of the others, in which case the required average (for a trimer) simplifies to

$$[\bar{M}]^{\text{calcd}} = 0.50[M]^{\text{calcd}}_{\text{gt,gt,gt}} + 0.50[M]^{\text{calcd}}_{\text{tg,tg,tg}}$$

This assumption was tested for two trimer linkage conformations by comparing the average of the gt,gt,gt and tg,tg,tg trimers with the average of all eight possible conformers. In both cases the difference was <1%.

For each linkage conformation we calculated the residue rotation of a dimer and of a trimer. We took the residue rotation appropriate to a long chain, $[M]_{\text{polymer}}$, to be $[M]_{\text{polymer}} = 3[M]_{\text{trimer}} - 2[M]_{\text{dimer}}$, where $[M]$ in all cases is a molar residue rotation. The polymer contains a single residue linked only at the C(1) position, a single residue linked only at the C(4) position, and all other residues, i.e., internal residues, linked at both positions. The above difference in calculated rotations thereby reflects the contribution from internal residues which dominates the rotation observed in polymers. The procedure represents a nearest-neighbor approximation to the interactions within the chain, which will be adequate for polysaccharides, where the linkages are well separated in space. For several conformations we tested the procedure with direct, and lengthy, calculations on all n-mers from n = 2–8. There was general consistency between the two procedures (see Results), which we took as justification for the simpler procedure.

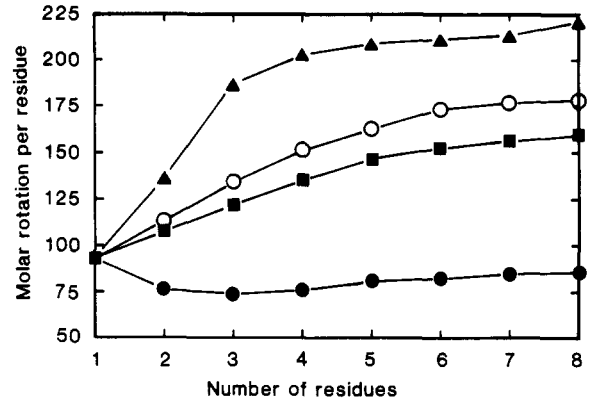

Figure 1. Calculated molar residue rotation of β-(1→4)-D-galactan as a function of chain length n, n = 1–8, for selected linkage conformations: (ϕ,ψ) = (−20°,−20°) (▲); (ϕ,ψ) = (20°,20°) (○); (ϕ,ψ) = (40°,40°) (■); (ϕ,ψ) = (60°,0°) (●).

Experimental Methods

Sample. The galactan was isolated from the cotyledons of the seeds of *Lupinus angustifolius*, cv. Unicrop as described by Hirst et al.[30] for *L. albus*. Acid hydrolysis and analysis of the neutral monosaccharides released by GLC of their alditol acetate derivative[31] gave the following composition for the polysaccharide: galactose, 85%; arabinose, 9%; xylose, 4%; rhamnose, 2%.

The Na_D specific rotation in aqueous solution was measured to be $[\alpha]_D$ = +74°. This result agrees with the value reported by Gerali and Bruno.[32] For a galactan residue weight of 162, $[M]$ = 120 deg cm^2 dmol^{-1}.

The contribution to the observed rotation from the non-galactose residues in the sample we used can only be estimated. From literature values of molar residue rotations,[33] a mixture of monomers having the same percent composition as our polymer sample is calculated to have only a slightly different rotation than pure galactose monomers, whether values for the free sugars or those for the pyranosides are considered. Qualitatively, this occurs because the molar rotations of galactose and arabinose are similar and those of xylose and rhamnose tend to cancel one another. For example, a mixture of free sugars is calculated to have a molar rotation of 91 deg cm^2 dmol^{-1} compared with 95 deg cm^2 dmol^{-1} for pure galactose. The contribution of the non-galactose residues to the linkage contribution cannot be estimated; it is presumed to be smaller than the overall uncertainty of the calculational method, estimated[1] to be ±24 deg cm^2 dmol^{-1}.

The VUCD spectrometer, described previously,[34] was operated with a spectral resolution of 3.2 nm, scan rate of 1.0 nm/min, and time constant of 100 s. The instrument was calibrated with camphorsulfonic acid; molar residue ellipticities are reported. The experimental uncertainty in solution spectra is estimated to be ±5%. Films were prepared by evaporation to dryness of the aqueous solution onto a CaF_2 window in a desiccator. Film thickness could be varied by adjusting the amount of solution deposited, but precise film thicknesses could not be measured because of thickness inhomogeneities. Films were rotated in the light beam by increments of 90° to detect any polymer orientation in the plane perpendicular to the light beam. Variations in the spectrum were within the experimental uncertainties in the film spectra of ±10% at 180 nm and ±20 at 150 nm.

Spectra were also obtained on a VUCD instrument at the U.S. National Synchrotron Light Source at Brookhaven National Laboratory in cooperation with Dr. J. C. Sutherland. The two instruments gave similar spectra; the signal-to-noise ratio is better with the synchrotron light source.

Results

Figure 1 illustrates, for selected linkage conformations, the convergences of the calculated molar residue rotation

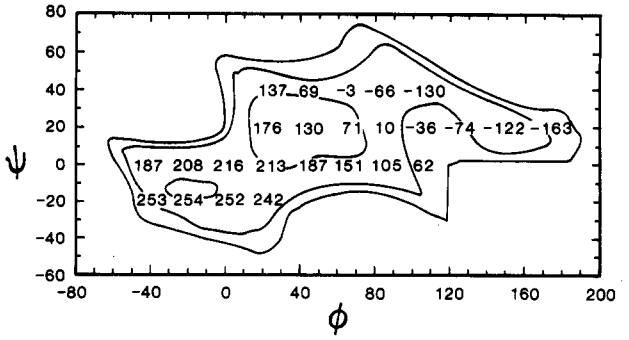

Figure 2. Calculated molar residue rotation, $[M]_{polymer}$, of β-(1→4)-D-galactan as a function of linkage conformation (ϕ,ψ), superimposed on a calculated potential energy contour map.[7] The two interior contours are at 0 kcal mol^{-1}, and the energy minimum of −1.7 kcal mol^{-1} is at approximately (ϕ,ψ) = (40°,20°).

Table I
Calculated Values of Molar Residue Rotation, [M], for β-(1→4)-D-Galactan Dimer, Trimer, Octamer, and Polymer[a]

(ϕ,ψ)	$[M]_{dimer}$	$[M]_{trimer}$	$[M]_{octamer}$	$[M]_{polymer}$
(−40°,0°)	128	148		187
(−40°,−20°)	154	187		253
(−20°,0°)	132	158		208
(−20°,−20°)	153	186	221	254
(0°,0°)	134	162	213	216
(0°,−20°)	150	184		252
(20°,40°)	96	110		137
(20°,20°)	113	134	170	176
(20°,0°)	129	157		213
(20°,−20°)	145	178	248	242
(40°,40°)	76	73	85	69
(40°,20°)	99	109		130
(40°,0°)	120	142		187
(60°,40°)	51	33	19	−3
(60°,20°)	79	76		71
(60°,0°)	107	122	160	151
(80°,40°)	24	−9		−66
(80°,20°)	57	41		10
(80°,0°)	92	96		105
(100°,40°)	−3	−41		−130
(100°,20°)	33	10		−36
(100°,0°)	73	69		62
(120°,20°)	11	−17		−74
(140°,20°)	−4	−43		−122
(160°,20°)	−9	−60		−163

[a] Rotations in deg cm^2 dmol^{-1}.

of β-(1→4)-D-galactan as the chain segment size is increased from one to eight residues; the units are deg cm^2 dmol^{-1}.

Table I includes the calculated molar residue rotations of β-(1→4)-D-galactan dimers and trimers for all of the linkage conformations examined. Also shown for each conformation is the molar residue rotation appropriate to a long chain, $[M]_{polymer}$, calculated as described above, and the molar residue rotation of the octamer for selected conformations.

Figure 2 displays values of $[M]_{polymer}$ superimposed on the calculated potential energy contour map of Brant.[7]

Figure 3 shows the VUCD spectrum of β-(1→4)-D-galactan in aqueous solution to 170 nm. A negative band is observed near 175 nm. Figure 4 displays the film spectrum. The negative dichroism observed near 175 nm we associate with the 175-nm band of the solution spectrum (Figure 3). At shorter wavelengths there is a larger negative band at 155 nm. In thin films the VUCD can be measured to 135 nm; in those spectra (not shown) there is a crossover near 145 nm and positive dichroism to the experimental cutoff.

Figure 5 shows the calculated VUCD spectra for β-(1→4)-D-galactan oligomers, n = 2–8, for the linkage conformation (ϕ,ψ) = (60°,40°).

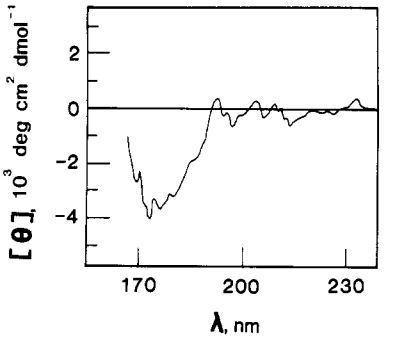

Figure 3. Measured VUCD spectrum of β-(1→4)-D-galactan in aqueous solution (c = 4 mg/mL, 100-μm path length).

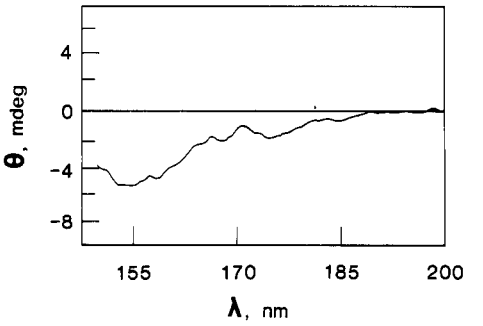

Figure 4. Measured VUCD spectrum of β-(1→4)-D-galactan film.

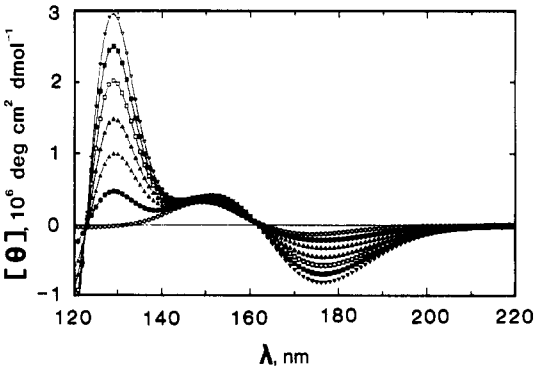

Figure 5. Calculated VUCD spectra of β-(1→4)-D-galactan n-mers, n = 2–8, for the linkage conformation (ϕ,ψ) = (60°,40°): n = 2 (○); n = 3 (●); n = 4 (△); n = 5 (▲); n = 6 (□); n = 7 (■); n = 8 (▽).

Discussion

Calculational Method. The method we use here has previously been applied only to monomers and disaccharides;[1,17-20] the assumptions and approximations in those applications have been discussed in detail.[1,17-20] Application to polysaccharides, reported here for the first time, requires an additional procedure to determine the residue rotation appropriate to an internal residue of a polymer chain. The experimentally observed molar residue rotation of 120 deg cm^2 dmol^{-1} is predominantly determined by internal residues. Our procedure of calculating the rotation of an internal residue by subtracting the dimer rotation from the trimer rotation, tested for some conformations, appears adequate but corresponds to a residue in which the linkage conformations preceding it and following it in the chain are the same; i.e., we examined only regular conformations. We keep this feature in mind when comparing the conformational dependence of calculated rotations with the experimentally determined rotation in solution.

The calculated residue rotation is sensitive to the conformation of the exocyclic hydroxymethyl group, as expected. From the results for the individual conformers (not shown in Table I) it was found that the optical rotation of the gt conformer, for the 50 dimer and trimer linkage conformations examined, is 70 ± 8 deg cm^2 dmol^{-1} more positive than that of the tg conformer. This is significantly close to the difference estimated with the use of empirically derived fragment parameters[29] and provides additional illustration of the correspondence between the present model and earlier empirical treatments of saccharide optical rotation.

The calculated results (Table I, Figure 2) display a substantial dependence of optical rotation on linkage conformation, as was also observed for cellobiose and maltose.[1] Rees and co-workers[35,36] have previously described an empirical treatment of the linkage contribution to saccharide optical rotation. It is not possible to extract from our results a "linkage" rotation that can be properly compared with Rees's linkage contribution, Λ. However, it is reasonable to expect a qualitative correspondence between the two descriptions, and one is clearly displayed in our present results. For example, as the linkage conformation is varied from (ϕ,ψ) = (0°,0°) to more positive ϕ or ψ values (Figure 2), the calculated rotation decreases and eventually becomes negative. Such behavior is expected also on the basis of the −120 (sin ϕ + sin ψ) term in Rees's expression for the conformationally dependent contribution to rotation.[35] Similarly, as ϕ and/or ψ become increasingly negative (Figure 2), the calculated rotation becomes increasingly positive, as likewise described qualitatively by Rees's expression.

The general qualitative similarity in the two approaches arises because both include many of the most important interactions involving the linkage geometry; e.g., the C(1)–O(1) bond interacting with the C(4′)–C(3′) and C(4′)–C(5′) bonds and the O(1)–C(4′) bond interacting with the C(1)–O(5) and C(1)–C(2) bonds. Our model also includes interactions of the C(1)–H and C(4′)–H bonds with their respective neighboring residues and interactions of the hydroxymethyl group with adjoining residues, albeit in an approximate manner. It would be difficult to establish which method gives a more accurate description of the details of the linkage conformation dependence, but it is satisfying that both methods of interpreting optical rotation lead to qualitatively similar descriptions of the dependence of rotation on linkage conformation.

The uncertainty in the calculated results has been described previously[1] and estimated to be ±24 deg cm^2 dmol^{-1}. In interpreting our calculated optical rotation dependence on torsional angles in oligosaccharides we are therefore justified in attributing significance only to optical rotation differences several times larger than this value. Because of the large conformation dependence we observe in the present case, the major conclusions of our analysis will be seen (below) to depend on large calculated differences.

Solution Conformation. The potential energy of β-(1→4)-D-galactan as a function of the linkage dihedral angles has been calculated by Brant using an empirically derived potential energy function;[7,16] it is shown in Figure 2. As is typical for polysaccharides, most of the (ϕ,ψ) conformation space is precluded by extreme steric overlaps. The allowed region includes a relatively broad range of ϕ-values because of the equatorial glycosidic bond. The axial aglycon bond, on the other hand, restricts ψ to a much smaller range of values, approximately described as $-40° \leq \psi \leq 40°$ for small ϕ-values and $\psi = 20°$ for large ϕ-values. Experience has shown that these general features are likely to persist even if other forms and parameterizations of potential function are used.

Details of calculated potential energy contours, on the other hand, often depend strongly on the energy function and on the simplifying assumptions usually made to specify hydroxyl and, particularly, hydroxymethyl conformations. One of the values of the present use of chiroptical properties is to provide some guidance in evaluating and further developing molecular modeling methods and their applications to polysaccharides.

The energy contours of Figure 2 display one major and one minor region of particularly favored conformations, all within 1.7 kcal mol^{-1} of the calculated energy minimum at approximately (ϕ,ψ) = (40°,20°). If these features persist in aqueous solution, they present a picture of rather unrestricted movement within the major region with occasional, or rare, excursions into the minor region depending on the detailed structure of the energy barrier connecting the two, not now reliably calculable. The "folded" conformation region near ϕ = 160° cannot be entirely discounted on energy grounds and is of some interest because it brings the O(5) atom into the range of favorable interactions with the C(3′) hydroxyl group, an interaction that plays an important role in cellobiose and, apparently, in maltose as well.[1]

The optical rotation experimentally observed in solution reflects a weighted conformationally averaged value, as do NMR chemical shifts, coupling constants, and NOE's. From one experimental parameter it cannot be determined whether there is a single rigid conformation or a rapidly interconverting set of conformations. Flexibility should be presumed unless there is compelling experimental evidence to the contrary.

The observed residue rotation of β-(1→4)-D-galactan in aqueous solution of 120 deg cm^2 dmol^{-1} is what is calculated for the energy minimum of Figure 2 at (ϕ,ψ) = (40°,20°), within the uncertainty of the calculation. Fluctuations about this linkage conformation tend to have opposite and canceling effects on the optical rotation, with positive increments in ϕ or ψ decreasing the rotation and negative increments having the opposite effect (Figure 2). On this basis, we interpret the results of the present calculations as indicating that the chain predominantly consists of residues in which the linkage geometry on either side is well described by (ϕ,ψ) = (40°,20°) and that there are fluctuations, albeit rather restricted, about this linkage geometry. The calculated potential energy contours and our conclusion based on optical rotation are consistent.

The optical rotation for conformations in the minor region near (ϕ,ψ) = (−20°,−20°) is calculated to be 100 deg cm^2 dmol^{-1} more positive than the observed value; we conclude that that region is populated only to a small extent, if at all. The minor region is not entirely energetically disallowed, largely on account of a favorable O(2)–O(3′) interaction. Conformations in the major region, however, are much less sterically hindered, as a simple molecular model clearly indicates, and are stabilized by the exoanomeric effect to the extent it is operative. Furthermore, the major region corresponds to the gt methoxy conformer of the monomeric methyl β-D-galactopyranoside, which is the favored conformer in solution.

We likewise conclude that the region near (ϕ,ψ) = (160°,20°) is also not populated significantly in aqueous solution; the calculated rotation for that region is more than 200 deg cm^2 dmol^{-1} more negative than the observed value.

Therefore, in all respects, the calculated potential energy contours and our analysis of the observed Na_D molar rotation are in agreement.

The negative CD band we observe in the VUCD solution spectrum (Figure 3) represents an n–σ^* transition and is

also observed in the monomeric methyl pyranoside.[37] It is too weak to contribute significantly to the Na_D molar rotation[38] and is not included in our calculational model of optical rotation. A 175-nm CD band is characteristic of saccharides, and a sector rule has been developed that rationalizes its sign for a large number of cases.[39] In terms of that sector rule, the observed negative 175-nm CD band is dominated by interactions within the galactose ring between the axial C(4) hydroxyl group and the O(5) chromophore. There are additional contributions from the C(2) hydroxyl group interacting with the O(1) oxygen atom which, in a polymer, would depend on linkage geometry. For the $(\phi,\psi) = (40°,20°)$ conformation, that contribution is also negative. The decreased intensity of the band we observe in the polymer (Figure 3), relative to that observed in the methyl pyranoside,[37] in light of the similar conformations, arises from perturbations present only in the polymer, a likely source being the hydroxymethyl group.

Film Conformation. In film samples, optical rotation, a dispersive phenomenon, is subject to experimental artifacts. On the other hand, film samples allow the CD to be measured much further into the vacuum-UV region. The $\sigma-\sigma^*$ transitions of saccharides incorporated into the present model give rise to CD components that are not experimentally accessible in solution; comparison with solution data (as above) requires conversion of the calculated CD to optical rotation via a Kronig–Kramers transform.

The VUCD band characteristically observed in polysaccharides near 155 nm (Figure 4) we have taken to represent the lowest energy $\sigma-\sigma^*$ transition, which in our model is typically calculated to appear near 170–175 nm (Figure 5). This excessive red shift in the calculated CD spectrum is inherent to the model.[17] Experience has shown that a calculated negative optical rotation is the result of a calculated negative lowest energy CD component. In the present case we therefore expect a correlation between the sign (and magnitude) of the calculated optical rotation (Figure 2) and the sign (and magnitude) of the lowest energy calculated CD band for all conformations. Figure 5 illustrates this correlation for the linkage conformation $(\phi,\psi) = (60°,40°)$; the calculated lowest energy $\sigma-\sigma^*$ CD band is negative as is the optical rotation (Figure 2). As ϕ or ψ is increased above these values, the lowest energy calculated CD band becomes increasingly more negative; as ϕ or ψ is decreased, the lowest energy calculated CD band becomes positive (compare Table I).

We conclude that the observed VUCD film spectrum, with its negative 155-nm band (Figure 4), reflects chain conformations in which $60° \leq \phi \leq 180°$ and $20° \leq \psi \leq 40°$. This range includes the conformation $(\phi,\psi) = (60°,40°)$, which is only slightly different from the solution conformation, a modification easily accounted for by packing interactions. A conformation near $(\phi,\psi) = (160°,20°)$ is, however, also expected to display a negative 155-nm VUCD band. The present study cannot discriminate between the two solid-state conformations. VUCD intensity measurements could distinguish the two; the calculated intensity increases with increasing ϕ. We have not yet developed a reliable method of intensity measurements for polysaccharide films, because of the extreme film thickness inhomogeneities. The method nevertheless puts limits on the range of conformations compatible with experimental VUCD measurements.

Acknowledgment. This work was partially supported by NIH Grant 24862 and NSF Grant CHE88-15167. The National Synchrotron Light Source, BNL, is supported by the U.S. Department of Energy, Division of Materials Sciences and Division of Chemical Sciences, under Contract DE-AC02-76CH00016.

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Registry No. β-(1→4)-D-Galactan, 9051-94-9.