trations may have less profound effects. The measurement of T_1's on high concentrations of sample, such as commonly used in natural abundance ^{13}C studies, may introduce aberrations due to intermolecular interactions and thus invalidate extrapolation to lower concentrations. When pH dependent studies are involved, complex changes in solution structure are likely which, in turn, will be reflected by a different behavior of the molecule.

As has been illustrated in this work, the behavior of a complex, nonrigid molecule such NAD^+ may be very different from that of a small molecule such as 1-methylnicotinamide, even though identical solution conditions were maintained.

Acknowledgment. The authors gratefully acknowledge financial support from the American Cancer Society, Grant BC-111, and the National Cancer Institute of the National Institutes of Health through Grant CA 15645, and helpful discussions with Dr. A. P. Zens.

References and Notes

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(2) Abbreviations: NAD^+: nicotinamide adenine dinucleotide; NAD^+-N^6-^{13}C, NAD^+-N^7-^{13}C: nicotinamide adenine dinucleotide with the carbon-13 label in the nicotinamide ring at position 6 or 7 (carbonyl), respectively.
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Optical Properties of Sugars. 3. Circular Dichroism of Aldo- and Ketopyranose Anomers[1a]

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Abstract: Techniques have been developed for measuring the circular dichroism spectrum of an individual sugar anomer. Vacuum ultraviolet spectra are presented for α- and β-D-glucose, α- and β-D-galactose, and α-D-xylose in D_2O to 165 nm. These spectra confirm our original suspicion that it is not meaningful to compare the circular dichroism spectra of sugars at anomeric equilibrium, even when the equilibrium mixtures have the same anomeric composition, because either anomer may contribute the bulk of the intensity to the spectrum of an equilibrium mixture. Instead, spectra of corresponding anomers should be compared. In addition, circular dichroism spectra of three ketopyranoses which do not undergo appreciable anomerization (α-D-sorbose, α-D-tagatose, and α-D-*manno*-heptulose) are presented to 165 nm. Difference circular dichroism spectra reveal similar contributions to the spectra of homomorphic and epimeric pairs when near-neighboring groups are the same. When near-neighboring groups are different, so are the difference circular dichroism spectra demonstrating the sensitivity of the technique to configuration and conformation. The low-energy band in the spectra of the aldohexoses is apparently due to the presence of the hydroxymethyl group. It is suggested that in solution, water structure about the sugar molecule may be more important than minor electrostatic interactions in determining the rotamer population of the hydroxymethyl group.

One expects the diverse biological activities of the carbohydrates and glycoproteins to be mediated by their conformation, in a manner analogous to nucleic acids and proteins. We have begun circular dichroism (CD) studies in the vacuum ultraviolet to try to understand the effects of configuration, sequence, and environment on carbohydrate conformation. In

Table I. Source and Optical Rotation of Aldo- and Ketopyranoses

	Source	Obsd $[\alpha]_D^{RT}$ (D_2O), deg		Lit. $[\alpha]_D^{20}$ (H_2O), deg		Ref
		Initial	Equil	Initial	Equil	
Aldohexopyranose						
α-D-Glucopyranose	Sigma	110.5	54.2	112.2	52.8	3
β-D-Glucopyranose	Sigma	17.8	53.3	18.7	52.8	3
α-D-Galactopyranose	Sigma	147.5	79.7	150.7	80.2	3
β-D-Galactopyranose	From α anomer	53.1	79.7	52.8	80.2	3
Aldopentopyranose						
α-D-Xylopyranose	Schwarz Mann	91.7	20.1	93.6	18.8	3
Ketohexopyranose						
α-L-Sorbopyranose (α-L-*xylo*-hexulose)	Pfanstiehl		−43.7		−43.4	3
α-D-Tagatopyranose (α-D-*lyxo*-hexulose)	Schwarz Mann		+2.3		−5.0	8
Ketoheptopyranose						
α-D-*manno*-Heptulopyranose	Pfanstiehl		28.5		29.4	21

the first paper in this series,[2] which recounted previous work in this area, we reported the CD of aqueous solutions of D-glucose, D-galactose, and D-xylose in the region of absorption for cyclic sugars. This was the first time Cotton effects had been measured for the cyclic forms of unsubstituted sugars. When such a monosaccharide is dissolved in a solvent, a complex equilibrium is established which results from reversible isomeric changes in the configuration of the group at C-1 (anomerization) and in the size of the ring (ring tautomerization).[3-5] We chose D-glucose, D-galactose, and D-xylose for our initial investigation of the vacuum uv CD properties of monosaccharides at equilibrium, because their equilibrium mixtures all contain approximately 65% β-D-pyranose and 35% α-D-pyranose. Both the α- and β-pyranoses of these sugars preferentially adopt the C1(D) conformation by Reeves' convention.[6] Measurements were made to 164 nm and the differences we observed for the signs and magnitudes of the CD bands were attributed to configurational and rotameric differences between the sugars.

However, in order to obtain information about the effects of configuration and conformation on monosaccharide CD by a comparison of such equilibrium spectra, even in the favorable case of compositional similarity, it is necessary to assume that the α and β anomers contribute rotational strength to the CD spectrum of their equilibrium mixtures in roughly the same proportion for each sugar. To avoid making this assumption and to obtain more spectral information we have developed a technique for measuring the vacuum uv CD of monosaccharide solutions which contain predominantly a single anomeric species. In this paper we present these measurements for the α- and β-pyranose forms of D-glucose and D-galactose and the α-pyranose form of D-xylose (Figure 1).

The CD spectra of the three ketopyranose anomers investigated, α-D-sorbose, α-D-tagatose, and α-D-*manno*-heptulose (Figure 1), were measured in water since these ketoses do not undergo appreciable isomerization upon dissolution.[7] These cyclic hemiketals have two oxygen-containing substituents attached to their anomeric carbon atom, a hydroxyl and hydroxymethyl group. Both the anomeric effect and steric considerations favor the anomeric configuration in which the hydroxymethyl group is oriented equatorially with the ring maintaining a C1 conformation.[8]

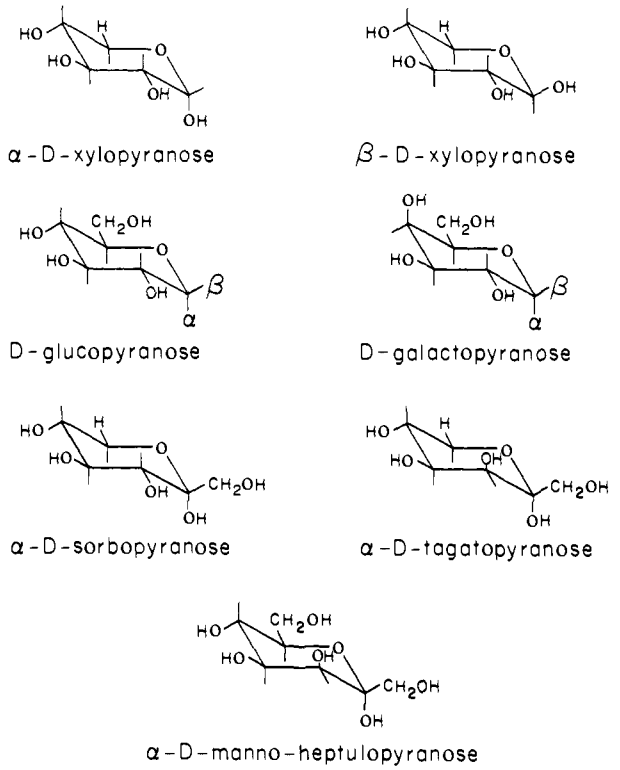

Figure 1. Configuration and predominant aqueous solution conformation of the sugars investigated. For the aldopyranose sugars, ring numbering begins with the anomeric carbon atom as C-1 and proceeds clockwise around the ring sequentially labeling each carbon atom. The hemiacetal ring oxygen takes the number of the proceeding carbon atom. The C-1 carbon atom of the α-ketopyranoses is exocyclic with the anomeric carbon atom C-2 and ring numbering proceeds as before. Numbers above 5 in the aldopyranose series and above 6 in the α-ketopyranose series refer to (additional) exocyclic carbon atoms.

Experimental Section

Materials. The source and rotatory properties of the five aldopyranose and three ketopyranose sugars investigated in this study are listed in Table I. Aldopyranose mutarotation was followed by observing the change in optical rotation with time in deuterium oxide (Bio-Rad, 99.8 mol % D_2O). The aldopyranose initial rotations were determined by extrapolation of the mutarotation measurements to time zero. β-D-galactopyranose was obtained from the α-anomer by Hudson's procedure.[9] β-D-Xylopyranose has never been crystallized.

Procedure. Mutarotation measurements were made to allow calculation of the sum of the rate constants for the opposing reactions (the mutarotation constant). Deuterium oxide was substituted for water as the solvent because mutarotation for aldopentoses in deuterium oxide is approximately threefold slower than it is in water at the same temperature.[3] Table II presents the mutarotation constant and isotope effect of the aldoses whose spectra were determined in this study. The last column gives the time necessary for the composition of a deuterium oxide solution of the particular sugar to change by 10%

Table II. Mutarotation Data for Aldopyranose Anomers Investigated

Aldopyranose	Mutarotational constant (D_2O, RT), m_1, min^{-1}	Isotope effect,[a] $m_1(H_2O, 20\ °C)$ to m_1 (D_2O, RT)	Time for 10% composition change, D_2O at RT, min
α-D-Glucopyranose	0.0020	3.18	39
β-D-Glucopyranose	0.0021	3.34	73
α-D-Galactopyranose	0.0026	3.08	25
β-D-Galactopyranose	0.0025	3.24	77
α-D-Xylopyranose	0.0064	3.12	11

[a] See ref 3.

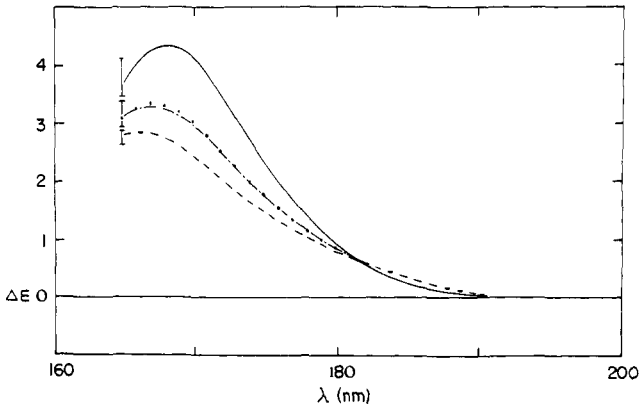

Figure 2. CD spectra of α-D-glucopyranose (——), β-D-glucopyranose (- - -), D-glucopyranose at anomeric equilibrium (- — -), and calculated equilibrium D-glucopyranose (· · ·).

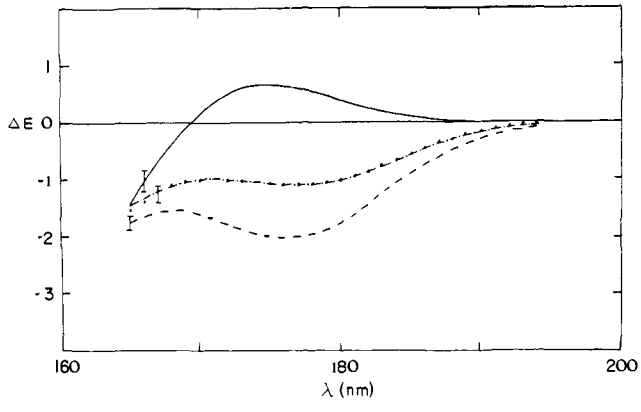

Figure 3. CD spectra of α-D-galactopyranose (——), β-D-galactopyranose (- - -), D-galactopyranose at anomeric equilibrium (- — -), and calculated equilibrium D-galactopyranose (· · ·).

from the value for the pure anomer at room temperature. The CD spectra of the anomeric aldopyranoses were measured before a 10% change occurred, although α-D-xylose spectra had to be recorded at 10 °C to achieve this.

Vacuum-dried samples were weighed and dissolved in degassed D_2O. With the exception of the measurements made on α-D-galactopyranose, CD spectra could often be rescanned before the anomeric composition of the equilibrating solution changed significantly. No photodecomposition was observed.

Quantitative spectra were measured in cells of known pathlength from 210 nm to about 172 nm and averaged. Qualitative spectra were measured from 210 to approximately 165 nm. At least three spectra were averaged and normalized to the measurements made in the cells of known pathlength. The bars on the reported spectra represent the reproducibility observed in the qualitative measurements. The precision of the quantitative measurements was about ±10%.

Other procedures and the cells used are described in paper 1[2] with the exception that Tetrasil S.E. (Quartz Products Corp., Plainfield, N.J.) or Suprasil 1 (Amerisil Inc., Hillside, N.J.) fused quartz windows were substituted for the CaF_2 windows used previously.

Apparatus. All specific rotations were measured on a Rudolph Model 80 polarimeter using a Rehovoth Instruments faraday cell and type A reader attachment for photometric detection. The CD spectrometer has been described elsewhere[10] and conditions of measurement are given in paper 1.[2]

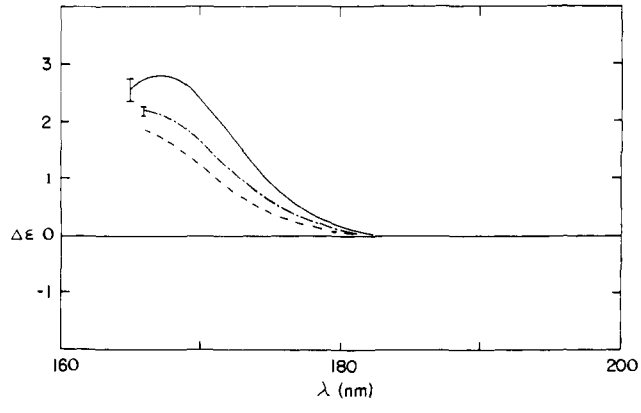

Figure 4. CD spectra of α-D-xylopyranose (——), D-xylopyranose at anomeric equilibrium (- — -), and calculated β-D-xylopyranose (- - -).

Results

The measured CD spectra for the α and β anomers as well as the observed and calculated equilibrium spectra of D-glucopyranose and D-galactopyranose are presented in Figures 2 and 3, respectively. The D-glucose equilibrium spectrum was calculated by adding 0.36 of the experimental α-anomer spectrum and 0.64 of the experimental β-anomer spectrum at each wavelength. This reflects the anomeric composition of equilibrium D-glucose solutions in deuterium oxide at 20 °C as determined from NMR measurement.[5] The D-galactose equilibrium spectrum was calculated similarly using 0.29 of the α- and 0.64 of the β-anomer spectra. In addition to the anomeric pyranoses, equilibrium solutions of D-galactose contain 0.07 parts furanose at 35 °C.[5]

Figure 4 presents the observed CD spectra for α-D-xylopyranose as well as the equilibrium mixture of anomers. Since the β-anomeric form of D-xylopyranose has not as yet been crystallized, the spectrum of this anomer was calculated by subtracting 0.35 of the α-anomer spectrum from the equilibrium spectrum. This is consistent with the anomeric proportions of xylose solutions determined at 0 °C.[4]

To confirm that we were measuring the CD spectra of pure anomers, we measured the mutarotation of α-D-glucose and α-D-galactose (which have short measuring times) in the CD spectrometer by observing the change in $\Delta\epsilon_{174}$ with time. The values obtained on extrapolation to zero time compared well with the values measured by continuous recording of the spectra. In addition, the observed equilibrium CD spectra of D-glucose and D-galactose are closely approximated by the composition weighted linear combinations of experimental anomer CD spectra (Figures 2 and 3). These results lead us to believe that we are measuring reasonable approximations to the pure anomeric aldopyranose spectra and that our calculated CD spectrum for β-D-xylose is correct.

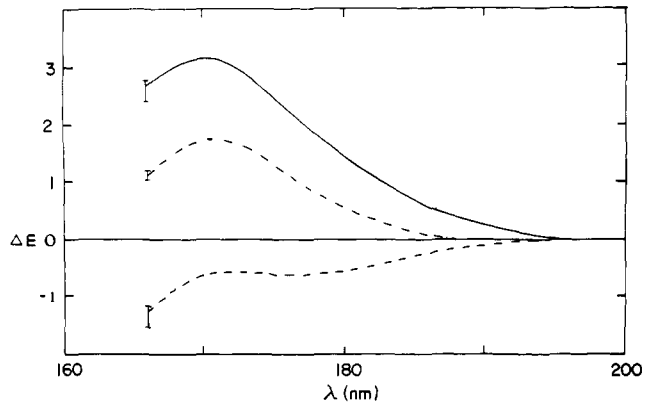

Figure 5. CD spectra of α-D-*manno*-heptulopyranose (——), α-D-tagatopyranose (- · · · -), and α-D-sorbopyranose (- - -).

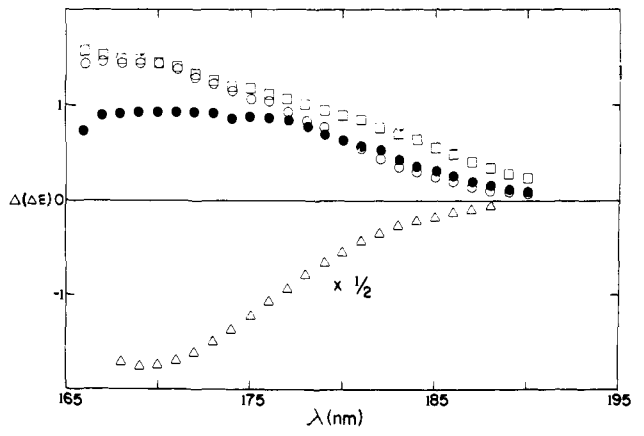

Figure 6. Difference CD spectra of homomorphic aldo- and ketopyranoses: (□) α-D-*manno*-heptulose minus α-D-tagatose; (○) α-D-glucose minus α-D-xylose; (●) β-D-glucose minus β-D-xylose; (△) α-D-sorbose minus α-D-xylose. Xylose spectra have been red shifted 1 nm before subtraction from glucose spectra and 3 nm before subtraction from sorbose spectrum in order to account for spectral shifts introduced by differences in solvent and temperature.

α- and β-D-xylose show only one clear CD band of moderate intensity in the region studied. The α anomer shows a positive band with a maximum at about 167 nm while the β anomer exhibits a positive band of somewhat lower intensity with a maximum just to the blue of 165 nm, the limit of solvent transmission. The CD bands for α- and β-D-glucose are similar to those for the corresponding xylose anomers but are more intense. Although their maxima are at about the same frequency as observed for the xylose sugars, the CD bands begin about 10 nm further to the red. This suggests the presence of a second low-intensity positive band which is about 10 nm to the red of the main band, but is seen only as a low-intensity tail at the red end of the spectrum.

The spectrum of the α anomer of D-galactose consists of a low-intensity positive band with wavelength maximum at about 174 nm. A second more intense and negative band has its maximum below 165 nm. In contrast, the first band for the β anomer is somewhat more intense and negative in sign with a maximum of 178 nm. It also has a second and more intense band with wavelength maximum below 165 nm. These first low intensity bands, which are clearly seen in the galactose sugars, presumably correspond to the low wavelength band which, as we have suggested, may be buried under the main transition for the glucose sugars. If this is the case, it would appear that the main band in the D-xylose and D-glucose spectra is either missing, blue shifted, or cancelled by large negative intensity in the D-galactose CD spectra.

The vacuum uv CD spectra for three ketopyranoses are presented in Figure 5. The α-D-sorbose spectrum was obtained for the enantiomorph, α-L-sorbose, and its sign reversed. This sugar exhibits a negative band of low intensity with wavelength maximum at about 177 nm as well as a more intense negative band whose maximum is below 165 nm. α-D-Tagatose is similar to α-D-sorbose, but the C-3 hydroxyl has changed from equatorial to axial. The onset of CD intensity is shifted about 7 nm to the blue and the compound exhibits a positive CD band of moderate intensity at about 171 nm. The long, low-intensity tail at the red end of the spectrum could be due to a second band. α-D-*manno*-Heptulose is similar to α-D-tagatose, but the C-6 carbon has a hydroxymethyl group as a substituent. The CD spectrum of this ketopyranose is similar to the α-D-tagatose spectrum but the positive band at about 170 nm is now considerably more intense and the onset of CD intensity is shifted 7 nm to the red giving a long, low-intensity tail.

Discussion

The principle of pairwise interaction has been introduced by Kauzmann et al.[11] as a method with a sound theoretical basis for relating optical rotatory properties to molecular structure. Most asymmetric molecules consist of chromophores which are not intrinsically dissymmetric but which exhibit CD bands because of interactions with the remainder of the molecule. If we divide a molecule into groups, then according to this principle, the optical activity is given as a sum of pairwise interactions between groups. In the case of monosaccharides, it is natural to divide the molecule into the chromophores themselves, the saccharide HC(OH)<, $-CH_2OH$, and -OCH(OH)- functional groups. The rotational strength of a CD band (which is related to the area under the band) will be the sum of contributions arising from all ways of pairing the functional groups in the molecule.

The pairwise principle suggests the use of CD difference spectra as a means of comparing the spectra of structurally related monosaccharides. This method has been applied previously to far-uv optical rotatory dispersion curves by Listowsky et al.[12] The difference spectra should reveal patterns and consistencies which might not be obvious from direct inspection of the spectra. It should be clearly understood that these difference spectra are not partial dichroisms associated with individual molecular centers but reflect the changes in group interactions between the two molecules compared.

Homomorphic sugars have the same configuration about each asymmetrically substituted carbon atom that is part of the pyranose ring but differ in the type of substituent attached to one carbon atom of the ring. Figure 6 presents the difference CD spectra of four homomorphic pairs which differ only in the presence of a hydroxymethyl group. Three related pairs, which differ at C-5 in the case of the aldopyranoses (or C-6 in the case of the ketopyranoses), show very similar difference spectra. This indicates that the rotamer distribution for the exocyclic hydroxymethyl group is similar for each of these three homomorphic pairs, as would be expected, since all have their C-4 hydroxyl groups oriented equatorially. Remembering that CD arises from the interactions between groups, we suggest the following. (1) The difference CD spectra may reflect the presence of an additional absorption band from the $-CH_2OH$ chromophore which becomes optically active through its interactions with the rest of the molecule. These interactions should be dependent on the conformational preference about the exocyclic bond. (2) The hydroxymethyl group may contribute to the observed CD only as a perturbing group. The similarity of the difference spectra suggests that the interactions between a perturbing hydroxymethyl group and the chromophores are much the same for the $-CH_2OH$ containing member of these three homomorphic pairs.

The similarity between the CD difference spectra of sugars which differ only in the presence of a hydroxymethyl group at

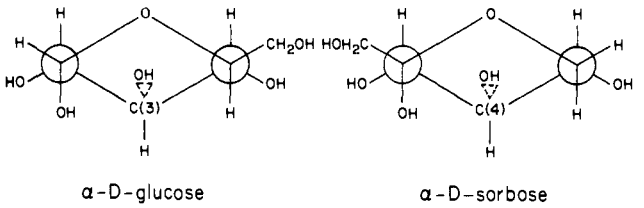

Figure 7. Newman projection of α-D-glucose and α-D-sorbose in the C1 conformation.

C-5 (C-6) supports the use of the pairwise principle. The fact that the difference spectrum for the homomorphic pair which differs at C-2 (Figure 6) is quite unlike the other difference spectra shows that different interactions can be expected to contribute diverse optical properties. Actually, this fourth difference spectrum might be expected to be of opposite sign under the pairwise approximation. Figure 7 shows that α-D-glucose and α-D-sorbose are nearly mirror images. All the interactions of the hydroxymethyl with the other groups are equal and opposite in sign for the two sugars, except for the interactions with the anomeric hydroxyls.[13]

Next we compare pairs of epimeric monosaccharides (Figure 8a) which differ from each other in the configuration about one asymmetrically substituted carbon atom of the pyranose ring. In all three of the epimeric pairs investigated, the orientation of the hydroxyl group adjacent to the hydroxymethyl is changed from equatorial to axial when the ring conformation remains C1. This type of change should depopulate the gauche-gauche (Figure 9) and populate the trans-gauche conformation of the hydroxymethyl group.[14] The difference spectra of Figure 8a thus reflect both the CD changes which result from an inversion of configuration at the ketopyranose C-3 or aldopyranose C-4 position and the effects of this inversion on the conformational distribution of the adjacent $-CH_2OH$ group.

Comparing the two D-galactose minus D-glucose difference spectra of Figure 8a indicates that the inversion of C-4 configuration results in a large negative change in the CD. It is apparent, however, that C-1 configuration does influence the CD change. The CD changes associated with configurational inversion at the ketopyranose C-3 position are of opposite sign.

A special class of epimers is the anomers which differ in configuration about the C-1 carbon atom, the only carbon atom bonded to two oxygen atoms. The difference spectra presented in Figure 8b show that the β to α anomerization of D-glucose, D-xylose, and D-galactose results in a positive contribution to their respective CD spectra. In addition, the similarity of the D-glucose and D-xylose difference spectra indicates that the anomerization of sugars belonging to the same homomorphic series results in parallel changes in conformation and electronic structure. As was the case for the C-4 epimers, the D-galactose difference spectrum indicates an electronic or rotameric difference between the α and β anomers.

Now let us consider the four points which we made in our previous discussion (paper 1). First, we pointed out that even if one is careful and only compares the CD spectra of equilibrium mixtures of anomers which have the same composition, still, one must make the tacit assumption that the two anomeric forms contribute rotational strength to their respective equilibrium CD spectra in roughly the same proportion for each sugar. In the equilibrium solutions of D-glucose and D-xylose the β anomer contributes about 1.5 times more rotational strength to the spectrum than does the α anomer whereas in equilibrium solutions of D-galactose the β anomer contributes five times more rotational strength than does the α anomer. Thus, even in favorable cases where the compositions of the equilibrium solutions are the same, any conclusions derived under the assumption of proportionate anomer contribution to the equilibrium spectra must be regarded as tentative.

Second, we suggested that there might be a second CD band in the low-energy region of the equilibrium D-glucose spectrum. Both α and β anomers of D-galactose have such a low-energy CD band, and one would not expect epimeric inversion to appreciably alter the energy of the chromophoric transitions. We suggest that both α- and β-D-glucose have positive bands, in analogy to the first galactose CD band in the long wavelength region of their CD spectra.

Third, we suggested that the first CD band, which is blue shifted or absent in the equilibrium D-xylose spectrum, was completely dependent on the presence of the C-5 hydroxymethyl group. The anomeric spectra provide no information on this point aside from the observation that both α- and β-D-xylose show the same blue shift in the onset of their CD.

Finally, our observations were consistent with the suggestion of Listowsky et al.[12] that the sign of the first CD band for equilibrium mixtures of D-glucose and D-galactose might be determined by the configuration at C-4 of the pyranose ring and its accompanying effects on the hydroxymethyl rotamer population (Figure 9). Specifically, a C-4 equatorial hydroxyl is expected to interfere with the trans-gauche rotamer and favor the gauche-trans. This hydroxymethyl conformation was presumed to be responsible for the positive CD of D-glucose. In contrast, the C-4 hydroxyl in galactose is axial which precludes the gauche-gauche.[14] The negative CD for galactose at anomeric equilibrium was interpreted to mean that the trans-gauche was preferred here. This idea is consistent with the CD spectra of the individual anomers of glucose (Figure

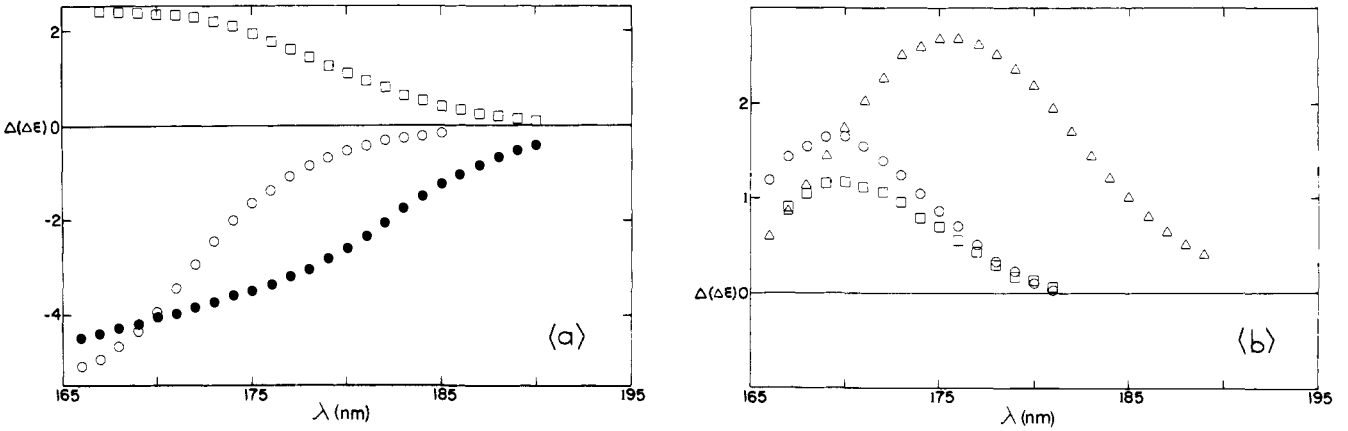

Figure 8. Difference CD spectra of aldo- and ketopyranose epimers: (a) (○) α-D-galactose minus α-D-glucose, (●) β-D-galactose minus β-D-glucose, (□) α-D-tagatose minus α-D-sorbose; (b) (○) α-D-glucose minus β-D-glucose, (□) α-D-xylose minus β-D-xylose, (△) α-D-galactose minus β-D-galactose. Xylose spectra have been shifted 1 nm.

2) and β-D-galactose, but the first band in α-D-galactose is positive. This is the cause of the anomalies in the CD difference spectra of Figures 8a and 8b.

If we consider what might cause the difference in sign between the CD spectra of the two anomers of galactose, we can think of two possibilities within the pairwise approximation. If we assume that there is no change in conformation, then the only unique interaction which is different between the two galactose-glucose epimeric pairs is that between groups attached to C-4 and C-1. This is a long-range interaction and is unlikely to be so large. In addition, it demands that the groups attached to at least one of these centers are chromophoric at wavelengths longer than 175 nm and our assignments in the following paper are at variance with this. Also, glucose does not show a change in sign of the first band on anomerization, as might be expected if this were the explanation.

Second, we can retain the idea that the sign of the first CD band is determined by the position of the hydroxymethyl group at C-5 and postulate that the orientation of this group is affected by solvent interaction with the hemiacetal group. This would mean that (C-5) $-CH_2OH$ conformation is strongly dependent upon both C-4 *and* C-1 configuration.

It is probably fair to say that the distribution of rotamers for the exocyclic hydroxymethyl group at C-5 in aqueous solution is still an open question. The rotamers found in crystals are probably determined by the best fit into the crystal structure consistent with maximizing hydrogen bonding. Distributions found in aprotic solvents may well not correspond to aqueous solution. Actually the energy differences among the three conformations are expected to be small so that solvent interactions may well determine the position of the hydroxymethyl group.[15]

Both thermodynamic[16-19] and hydrodynamic[20] measurements indicate that the saccharides are extensively hydrated in aqueous solution. Such strong solute-solvent association is a necessary prerequisite for hydroxymethyl conformation to depend on solvation. The 7.5-nm shift (Figure 3) in the onset of the first CD band between the two anomers of D-galactose suggests a difference in water structure and hydrogen bonding between these two sugars. Although more work is necessary, we suggest that such a difference in water structure and hydrogen affects the rotamer population of the hydroxymethyl in α- and β-galactose. The difference in sign for the first band in the CD spectra of the two anomers may well be a result of a difference in rotamer population.

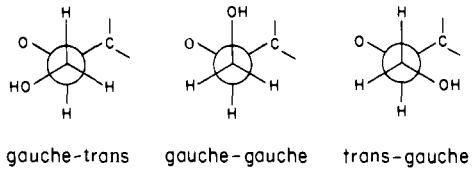

Figure 9. Staggered conformations for the CO bond of a hydroxymethyl group. The labeling refers first to the exocyclic conformation with respect to the ring CO bond and second with respect to the ring CC bond. This view is for aldohexopyranose C-6,O-6 bonds or ketoheptulopyranose C-7,O-7 bonds. Labeling is defined analogously for 2-ketopyranoses, but the conformations are enantiomorphic since the CO and CC bonds are reversed.

References and Notes

(1) (a) This work was supported by National Science Foundation Grant No. BMS74-01533; (b) NDEA Title IV Graduate Fellow; (c) Recipient of Public Health Service Research Career Development Award GM-32784 from the Institute of General Medical Sciences.
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(13) Both sugars would be expected to have the same (but mirror image) hydroxymethyl rotamers since both have adjacent equatorial hydroxyls. Note that all these arguments are equally true for α-D-galactose and α-D-tagatose.
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