Optical Properties of Sugars. I. Circular Dichroism of Monomers at Equilibrium[1]

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Abstract: Vacuum-ultraviolet circular dichroism spectra are presented for the aldohexoses D-glucose and D-galactose, and the aldopentose D-xylose in aqueous solution to 1640 Å. This is the first time Cotton effects have been measured for unsubstituted monosaccharides. These three sugars were chosen for our preliminary investigation because the similar composition of their equilibrium mixtures makes comparisons possible. Spectra of these monosaccharides at equilibrium reveal differences in sign and magnitude which are attributable to the configurational differences of the sugars. For these sugars, the sign of the first circular dichroic band is apparently determined by the configuration at C-4 of the pyranose ring and its effect on the conformation of the hydroxymethyl at C-5. An axial C-4 hydroxyl and its associated C-5 conformation result in a negative first band, while an equatorial C-4 hydroxyl and its associated C-5 conformation result in a positive first band. The dangers of investigating equilibrium mixtures of monosaccharides are discussed.

Optical rotation has played an important role throughout the long history of saccharide stereochemistry. With the advent of current instrumentation, optical rotatory dispersion (ORD) and circular dichroism (CD) measurements to 1850 Å have become possible. Such measurements have provided valuable information for many mono-, oligo-, and polysaccharides containing chromophores which absorb in the visible or ultraviolet region.[4-10] Stone[7] was able to correlate ORD spectra with structural differences in the linkages of the mucopolysaccharides heparin and chondroitin sulfate C. Beychok and Kabat,[4] using the octant rule[11] to interpret variations in ORD, were able to infer preferred alignments of the planar amide group in the 2-acetamido-2-deoxy sugar constituents of milk and blood group oligosaccharides. Listowsky, Avigad, and Englard,[9] by analysis of the CD spectra of muramic acids, were able to suggest that the lactyl moiety of muramic acid is of the D configuration.

However, studies of unsubstituted polysaccharides have been limited by available instrumentation, since absorption bands in these sugars occur below 1850 Å. To date workers have only been able to study the plain dispersion and CD curves found above 1850 Å, and these yield little information. Nevertheless, Listowsky, Avigad, and Englard[12,13] and Tanford, Pace, and Davidson[14] have had remarkable success in their study of unsubstituted monosaccharides by far-ultraviolet CD and ORD. They have shown that by judiciously selecting monosaccharides for comparison, information about configurational and conformational effects on the optical activity of the compounds can be had, even from these few data.

With the recent development of a CD spectrometer for the vacuum-uv region by one of the authors,[15] measurements on unsubstituted sugars have now become feasible. This paper describes the vacuum-uv CD properties of equilibrium mixtures of three aldopyranoses, D-glucose, D-galactose, and D-xylose. These specific monosaccharides were selected because of the similarity in the composition of their equilibrium mixtures, and because the conformations of their six-membered ring are clearly defined.[16] Clear interpretation of observed bands awaits the measurement of the individual spectra of the anomeric forms.

Experimental Section

Materials. The D-glucose and D-xylose were commercial samples of high purity supplied by Sigma and Mann Research Laboratories, respectively. The D-galactose was the "substantially glucose free" grade supplied by Sigma. Their equilibrium specific rotations at the sodium D line corresponded to reported literature values.[17]

Procedure. All solutions were freshly prepared using glass-distilled water, and ample time was allowed for equilibration between the anomeric forms. The measurements were performed over a concentration range from 0.1 to 2.0 *M*. We were concerned about possible artifacts due to solute–solute or solute–solvent interaction at these high concentrations, but the spectra recorded were found to be independent of concentration over this range. Also, the activity coefficient of glucose is near unity even for this high range of concentrations.[18]

We kept the total optical density of the cell, solvent, and solute below 1.0 to prevent artifacts. Three types of cells of differing path length were used to overcome the high water absorptivity in this region. Commercially available cylindrical quartz cells of 63-μ path length were used effectively to 1780 Å. Variable-path-length cells were useful to approximately 1700 Å with a 5-μ spacer. The path length was determined interferometrically[19] before each use. To obtain CD curves to 1640 Å, we placed a small drop of sample between clean CaF_2 windows with no spacer. The windows were

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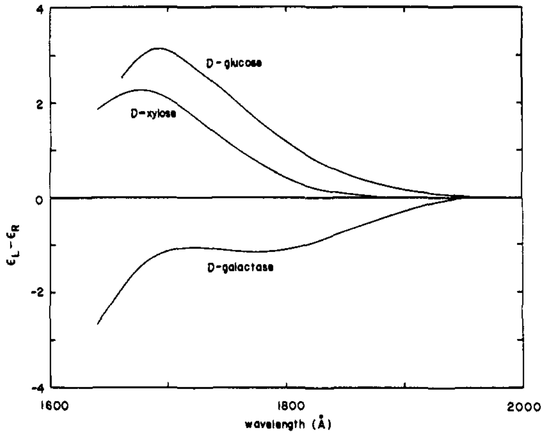

Figure 1. Circular dichroism spectra in aqueous solution for equilibrium mixtures of three closely related sugar monomers. The composition of the equilibrium mixtures of each of these sugars is similar.

choosen so that only a single fringe was observable over most of the area of the windows. No flattening of CD bands, the expected artifact for wedge-shaped path lengths, was observed. The path length was not determined, but the curves were normalized to measurements in the other two cells.

Apparatus. The vacuum-ultraviolet CD spectrometer used in this study has been described elsewhere.[15] Spectra presented here were recorded with a 16-Å spectral slit width, 10 or 30 sec time constants, and at corresponding scan rates of 10 or 5 Å/min, respectively. The spectrometer was calibrated taking $\epsilon_L - \epsilon_R = 2.20$ at 2905 Å for *d*-10-camphorsulfonic acid in water.[20] CD measurements on numerous compounds agree within experimental error with those taken to 1900 Å on commercial instruments.

Results

The physical properties of a sugar in solution are determined by the predominant conformations of that sugar and weighted with respect to the equilibrium distribution of the sugar between ring (furanose–pyranose) and anomeric (α–β) forms. For this reason, comparisons of optical properties require a careful selection of the sugars to be studied. We have chosen D-glucose, D-galactose, and D-xylose for our initial investigation, since (1) all three monosaccharides contain very little furanose in their equilibrium solutions;[16] (2) they exist in nearly the same proportion of anomers in solution;[21] and (3) the conformation of all six anomers is Cl[22,23] by Reeves' convention.[25,26] The three sugars are also structurally related. Xylose differs from glucose only in the absence of a hydroxymethyl group at position C-5 of the pyranose ring, whereas galactose differs from glucose only in that the hydroxyl at C-4 is axial rather than equatorial.

The vacuum-uv CD spectra of these compounds are presented in Figure 1. The three spectra are the average of a number of determinations for each sugar. D-Glucose shows a positive dichroism centered at 1695 Å with $\epsilon_L - \epsilon_R = 3.2$; similarly, D-xylose has a positive dichroic band centered at 1675 Å with $\epsilon_L - \epsilon_R = 2.25$. In contrast, D-galactose shows two negative bands, the first occurring at 1770 Å with $\epsilon_L - \epsilon_R = 1.2$, and a second larger band centered at wavelengths below 1640 Å.

One is tempted to find a second band in the long red tail of the D-glucose spectrum. Indeed, the shape of the D-xylose spectrum is very different from that of D-glucose; *i.e.*, although these two bands have their transition maxima only 20 Å apart, their low-energy envelopes begin 100 Å apart. Conversely, the D-glucose and D-galactose dichroic curves begin at approximately the same energy, but the first band in D-galactose peaks 80 Å to lower energy of the first band in D-glucose. These observations lead us to suspect that there is a band, in analogy to the first band in D-galactose, hidden under the long red tail of D-glucose.

Discussion

Cyclic ethers and simple alcohols are model compounds which contain the basic chromophores found in sugars. Vacuum-uv absorption spectra of these compounds in the vapor phase indicate the presence of two bands for the ethers and one for the alcohols in the region above 1667 Å.[27,28] For tetrahydropyran in the vapor phase, these bands are located at about 1890 and 1730 Å and have been attributed to the excitation of the unshared electrons of the pyranoid oxygen.[27] The bands we observe in the CD spectra of these sugars are all to the blue of the first vapor-phase bands of the model ethers and alcohols. Transitions involving nonbonding electrons are often blue shifted on going from the vapor phase to protic solvents.[29]

In addition, the C-1 carbon of the pyranose ring (the anomeric carbon) is unique in that it is attached to two oxygen atoms. A strong interaction undoubtedly exists here, so that in sugars we must also deal with a new chromophore, the H_1–O_1–C_1–O_5–C_5 hemiacetal group.

At this time it is not possible for us to assign the observed CD bands to any particular chromophore, although they probably involve the nonbonding electrons on oxygen. Since the first vapor-phase bands of ethers and alcohols occur at approximately the same energy,[28] the first CD band of the monosaccharides may be due to a number of nearly degenerate transitions, and the identity of the lowest energy band may well vary from sugar to sugar.

Glucose and galactose differ only in the configuration of one hydroxyl, yet their CD spectra are vastly different. In agreement with Listowsky, Avigad, and England's far-uv optical activity studies,[12,13] we find the gluco configuration (C-4 hydroxyl equatorial) to be associated with a positive rotational strength and the galacto configuration (C-4 hydroxyl axial) with a negative rotational strength. Apparently, the sign of the first CD band in these sugars is determined by the configuration at C-4 of the pyranose ring and its influence on the free rotation of the hydroxymethyl group at C-5.[30] Of course, the sign of the first band cannot be determined by the configuration at C-2 or C-3, since these are the same for both galactose and

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[30] This statement should not be generalized to the whole diastereomeric series.

glucose. However, the role of the optically active center at C-1 deserves further consideration. When one compares the CD of equilibrium mixtures, he must make the tacit assumption that the two anomers contribute rotational strength in roughly the same proportion for each sugar. This is true even if the equilibrium mixtures have the same composition. For instance, it is possible that the α anomer of glucose contributes the entire CD spectrum for the equilibrium mixture, while the β anomer contributes the entire spectrum for galactose. Our interpretation of the spectra, as has that of previous workers, rests on the above assumption.

Figure 2 shows the three possible staggered conformations of the exocyclic hydroxymethyl group. Glucose probably prefers conformation I (indeed it does in the solid state[31-34]) which alleviates the unfavorable diaxial and peri interactions of II and III. Galactose, on the other hand, probably prefers conformations I and III, II being ruled out because of severe "nonbonded" interactions associated with the C-4 and C-6 diaxial hydroxyls. Everything being equal, III may be the favored conformation, since it maximizes the angle between the C_5–O_5 and C_6–O_6 permanent dipoles. Thus, as has been suggested by Listowsky, Avigad, and Englard,[12,13] it appears that the C-5 hydroxymethyl group, when aligned as in I, determines a positive sign for the first dichroic band in D-glucose, while an alignment such as III determines a negative band for D-galactose.

Indeed, if one accepts the hypothesis that there is a CD band in the long red tail of the glucose spectrum, as discussed in the Results section, then we can go one step further. Since the first CD band has either disappeared or been strongly blue shifted in xylose, which does not contain the C-5 hydroxymethyl, it is clear that this band in glucose is completely dependent on the hydroxymethyl group.

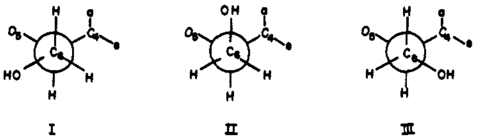

Figure 2. The three possible staggered conformations of the exocyclic hydroxymethyl group. The C-4 hydroxyl is axial (a) for galactose and equatorial (e) for glucose. This is a view down the bond from C-6 to C-5.

In this preliminary work we have investigated the CD properties of equilibrium mixtures of monosaccharides in the vacuum-uv region. However, since equilibrium mixtures of different sugars usually vary greatly in composition, a comparison of the optical properties of such mixtures is probably not meaningful. Indeed, even when the compositions are the same, the assumption pointed out above must still be made. In order to avoid the difficulties inherent in equilibrium mixtures, it would seem profitable to study the methyl glycopyranosides, since the configuration at C-1 is fixed. However, initial work in this laboratory with methyl α- and β-glucopyranoside indicates that the introduction of the acetal linkage results in spectral properties which are quite different from those of the free sugar. A study of the glycosides would then appear to be a separate problem. Therefore, we feel that in order to obtain more valuable information on free sugars one must study the optical properties of the individual anomers. Such a study is now being initiated in this laboratory. It should elucidate the effects of the configuration at each asymmetric carbon as well as the ring conformation on the CD properties of monosaccharides. With such information available, it should be possible to empirically predict saccharide configuration and conformation from the sign and intensity of the various vacuum-uv CD bands.

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