Optical Properties of Sugars. 4. Circular Dichroism of Methyl Aldopyranosides[1a]

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Abstract: Circular dichroism spectra in the vacuum ultraviolet to 165 nm are presented for aqueous solutions of 12 methyl aldopyranosides. Difference spectra of homomorphic and epimeric pairs reveal that the circular dichroism of these sugars bears more similarity to the spectra of aldopyranoses than is initially apparent. For a given anomer, changes on going from the pyranose to the corresponding methyl pyranoside are very similar. Also, changes in circular dichroism spectra which occur when a hydroxymethyl group is added to C-5 are similar for corresponding pyranose and methyl pyranosides. These difference spectra verify the use of the pairwise principle and show that the methyl pyranosides investigated have the same conformation as the corresponding pyranoses where comparisons are possible. With these data it is possible to tentatively assign specific chromophores to the first three bands in methyl pyranosides as well as the first band in the pyranoses. Apparently, the first band (~185 nm) in methyl pyranosides is due to the ring oxygen, the second (~175 nm) to the methoxy group, and the third (below 165 nm) at least in part to the methoxy group. The signs of the second and third bands are correlated with configuration about the anomeric carbon. The first band in the pyranoses (~180 nm) is apparently due to the ring oxygen. The ring oxygen transition is red shifted considerably when a hydroxymethyl on C-5 or methoxy group on C-1 shields the chromophoric nonbonding electrons from the hydrogen-bonding solvent.

Methyl aldopyranosides, unlike the aldopyranoses studied in our third paper[2] in this series, do not undergo mutarotation upon dissolution. Having a fixed conformation at C-1 has the advantage that it simplifies circular dichroism (CD) measurements, but these molecules have a methyl acetal group whose optical properties are expected to be different from those of the hemiacetal found in the aldopyranoses. Here we report the CD spectra of 12 methyl aldohexo- and pentopyranosides in aqueous solution to about 165 nm (Figure 1). We investigate the effects the O-5, C-1, O-1 system has on the spectra of the simple monosaccharides and obtain additional experimental data on the CD changes associated with anomerization, epimerization, and the addition of a hydroxymethyl group in homomorphic pairs of sugars.

All but two of these pyranosides predominantly adopt the usual C1 chair conformation according to Reeves' classical study of stereospecific complex formation with cuprammonium reagent.[3] Reeves' assignments are corroborated by empirical[4,5] and semiempirical[6] free-energy calculations as well as H-1, H-2 coupling constants[7] for the majority of the sugars that we have investigated. Aqueous solutions of the two other pyranosides studied, β-D-riboside and α-D-lyxoside, are believed to contain substantial amounts of both the C1 and 1C conformers.[8,9]

The optical rotatory dispersion (ORD) of anomeric pairs of tetrahydropyranyl ethers, 2-deoxy- and 2,6-dideoxy-D-glycopyranosides, have been reported by Klyne et al. to 200 nm.[10] They proposed that the plain dispersion curves they observed in the far-uv were due to the acetal chromophore since the sign of the rotation was directly correlated with the anomeric configuration. Listowsky et al.[11] investigated the ORD of a number of methyl glycosides to 185 nm. They suggested that the optical rotation in this region is predominantly associated with ring oxygen absorption and that the sign and magnitude of the rotation are determined primarily by the stereochemical arrangement of groups about this oxygen atom. Subsequently, Listowsky and Englard[12] measured the CD spectra of several monosaccharides to 188 nm. Their results showed that the sign of the first CD band did not depend on the configuration about the anomeric carbon. This tended to confirm the idea that the first band was due to the ring oxygen. The data presented here allow us to tentatively assign CD bands in the spectra of the methyl pyranosides to specific chromophores. Finally, these conclusions allow us to assign chromophoric groups to CD transitions for the pyranoses.

Experimental Section

Materials. The source and rotatory properties of the 12 methyl aldopyranoside sugars studied are listed in Table I. Three aldopyranosides (methyl α-D-lyxopyranoside, methyl α-D-ribopyranoside, and methyl 2-deoxy-α-D-glucopyranoside) were synthesized in our laboratory from their corresponding aldopyranoses following the general technique of Cadotte et al.[13]

Procedure and Apparatus. These details are the same as in previous work.[2]

Results

CD spectra for the 12 methyl aldopyranosides in aqueous solution are presented in Figures 2–8. The spectrum of methyl α-D-xyloside (Figure 2) shows that at least three transitions contribute to its CD spectrum between 200 and 165 nm. The sugar has a very weak negative band at about 185 nm, a weak positive band at about 174 nm, and a more substantial negative band which peaks below 165 nm. Presumably the CD spectrum of methyl β-D-xyloside also consists of three CD bands. The long-wavelength band is again negative and is seen as a long, very low intensity tail on the second CD band. The second and third bands are similar in intensity but opposite in sign to those found for methyl α-D-xyloside. The signs of these two short wavelength bands are correlated with the configuration about the anomeric center for all the methyl aldopyranoside spectra. This suggests that the chromophore responsible for these bands must be in at least approximately enantiomorphic environments in the α and β anomers.

The CD spectrum of methyl α-D-glucoside (Figure 3) also contains at least three CD bands. In contrast to methyl α-D-xyloside, the first band is positive and appears as a low-intensity tail at the long wavelength end of the spectrum. The second and third bands are similar to those of the xyloside but increased in intensity. Methyl β-D-glucoside only clearly exhibits two bands, a positive low-intensity band at 182 nm and a more intense positive band whose maximum lies below 165 nm. Of course the CD spectrum could well involve three or more component CD bands. Certainly one does not expect the addition of a hydroxylmethyl group at the C-5 carbon of a methyl xyloside to decrease the number of CD bands present.

While the CD spectra of the methyl glucosides are predominantly positive, changing the C-4 hydroxyl from equatorial to axial to produce the methyl D-galactosides results in

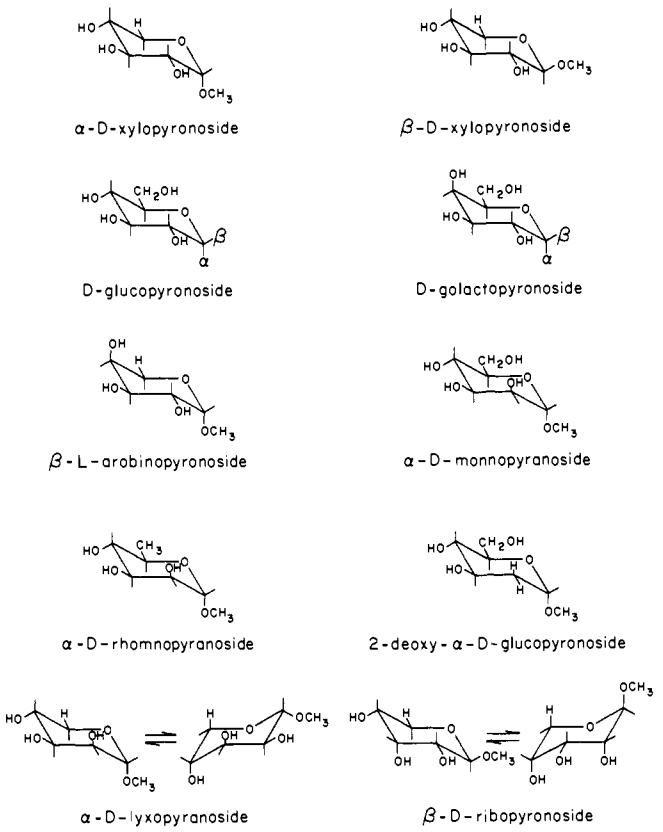

Figure 1. Configuration and predominant aqueous solution conformation of the sugars investigated. Ring numbering begins with the anomeric carbon atom as C-1 and proceeds clockwise around the ring sequentially labeling each carbon atom. The acetal ring oxygen takes the number of the proceeding carbon atom.

CD spectra which are predominantly negative (Figure 4). The α anomer has a low-intensity negative band at about 185 nm and a second very intense negative band with a maximum below 165 nm. The β anomer shows an intense negative band at about 173 nm with a long wavelength tail which could well be a second band. Clearly the spectra of these six compounds differ greatly in magnitude and shape from the CD spectra of corresponding pyranoses presented in paper 3.

L-Arabino sugars in the C1(L) conformation are homomorphic to D-galacto sugars in the C1(D) conformation. The spectrum of methyl β-L-arabinoside (Figure 5) is very similar to that of methyl α-D-xyloside and shows the effects of at least three CD bands above 165 nm. This similarity indicates that C-4 epimerization itself, at least when the anomeric methoxyl group is oriented axially, has only a small effect on the first two

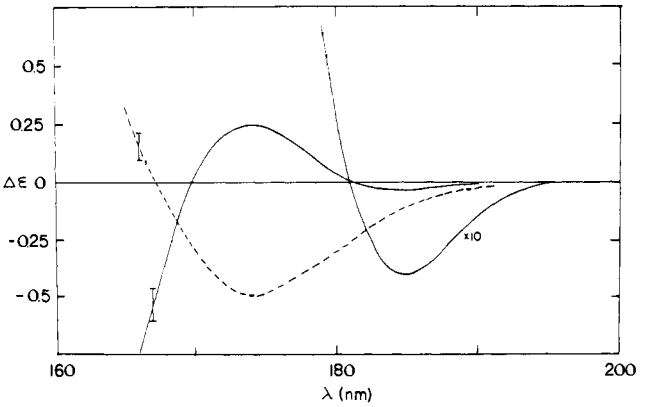

Figure 2. CD spectra of methyl α-(—) and β-(- - -) D-xylopyranoside.

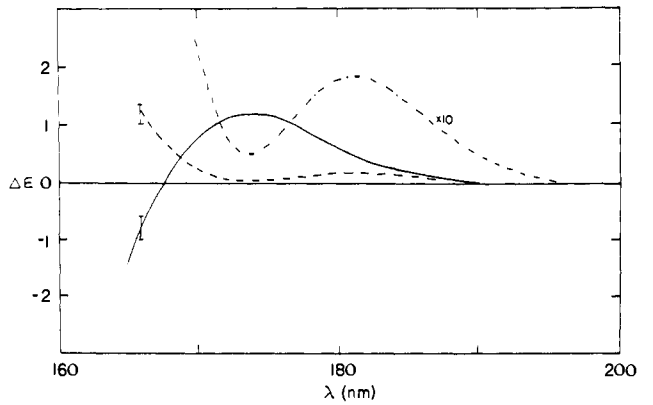

Figure 3. CD spectra of methyl α-(—) and β-(- - -) D-glucopyranoside.

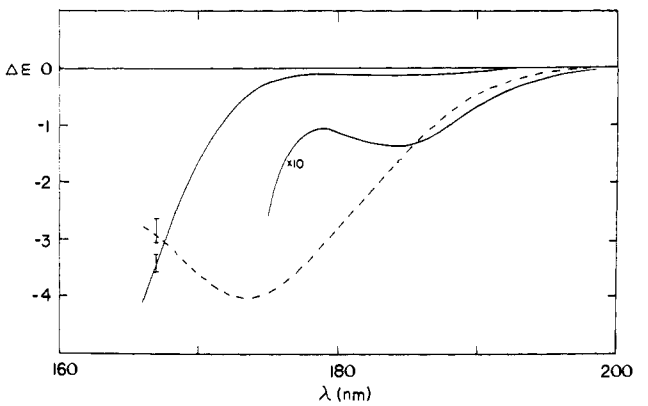

Figure 4. CD spectra of methyl α-(—) and β-(- - -) D-galactopyranoside.

Table I. Source and Optical Rotation of Methyl Aldopyranosides

	Source	Obsd $[\alpha]^{RT}D$ (H_2O), deg	Lit. $[\alpha]^{20}D$ (H_2O), deg	Ref
Methyl aldohexopyranoside				
Methyl α-D-glucopyranoside	Sigma	158.8	157.9	25
Methyl β-D-glucopyranoside	Pfanstiehl	−32.4	−32.5	25
Methyl α-D-galactopyranoside	Pfanstiehl	190.8	192.7	25
Methyl β-D-galactopyranoside	Pfanstiehl	−1.2	−0.4	25
Methyl α-D-mannopyranoside	Pfanstiehl	77.9	79.0	25
Methyl α-L-rhamnopyranoside	Koch-Light Labs	−62.8	−62.5	25
Methyl 2-deoxy-α-D-glucopyranoside	Synthesized	134.0	135.0	16
Methyl aldopentopyranoside				
Methyl α-D-xylopyranside	Pfanstiehl	154.2	153.9	25
Methyl β-D-xylopyranoside	Pfanstiehl	−64.9	−65.5	25
Methyl β-L-arabinopyranoside	Koch-Light Labs	242.5	245.5	25
Methyl α-D-lyxopyranoside	Synthesized	60.0	59.4	14
Methyl β-D-ribopyranoside	Synthesized	−102.6	−105.0	15

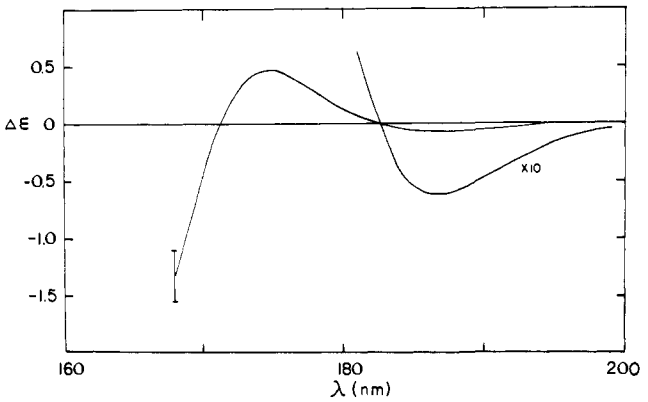

Figure 5. CD spectrum of methyl β-L-arabinopyranoside.

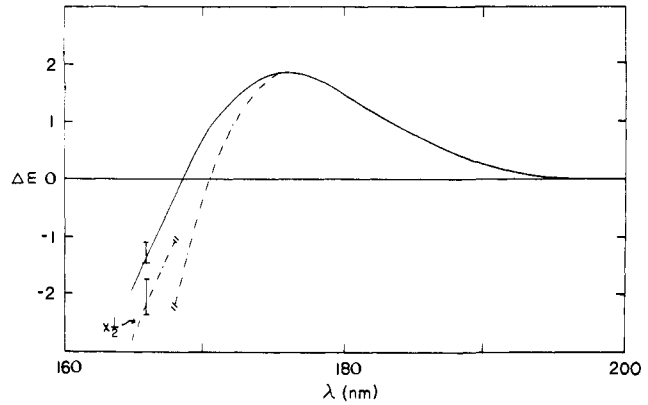

Figure 6. CD spectra of methyl α-D-mannopyranoside (—) and methyl α-D-rhamnopyranoside (- - - -).

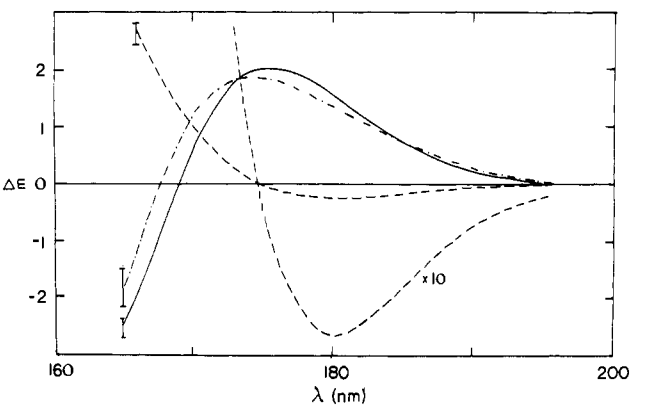

Figure 7. CD spectra of methyl α-D-lyxopyranoside (- - - -), methyl 2-deoxy-α-D-glucopyranoside (—), and methyl β-D-ribopyranoside (- - -).

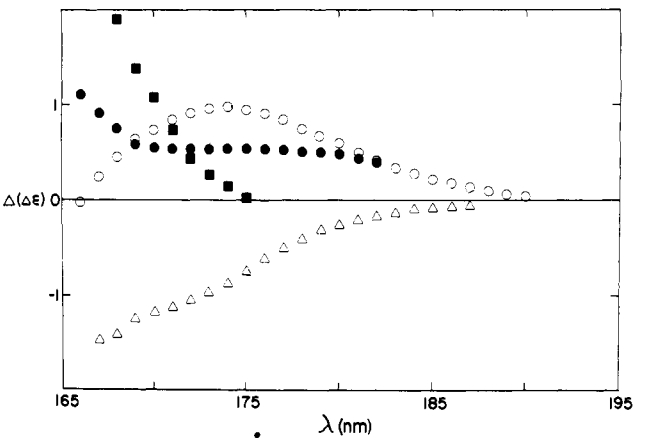

Figure 8. Difference CD spectra of homomorphic methyl aldopyranosides: (○) β-D-glucoside minus α-D-xyloside; (●) β-D-glucoside minus β-D-xyloside; (△) α-D-galactoside minus β-L-arabinoside; (■) α-D-mannoside minus α-D-rhamnoside.

CD bands. Comparing the methyl glucoside and galactoside spectra, this in turn suggests that the effects of epimerization on the conformational distribution of an adjacent hydroxymethyl group must have a significant influence on the signs of the first two CD bands.

The CD spectra of three members of the lyxo configurational series are presented in Figures 6 and 7. The spectrum of the rhamnoside was obtained with the enantiomorph and its sign reversed. These three sugars, methyl α-D-mannoside, methyl α-D-rhamnoside, and methyl α-D-lyxoside all have a positive CD spectra with a simple shape. The onset of intensity is 195 nm with a long tail. There is a positive peak of moderate intensity at about 176 nm while the effects of a band below 165 nm quickly send the curve negative. As stated earlier methyl α-D-lyxoside is known as a mixture of the C1 and 1C conformations at room temperature in aqueous solution. The sugar is probably greater than 70% in the usual C1 conformation but it is possible that the minor conformer contributes the bulk of the intensity to the equilibrium spectrum.

Methyl 2-deoxy-α-glucoside (Figure 7) is a derivative related to both methyl α-D-glucoside and methyl α-D-mannoside. Its CD spectrum is very similar to those of the lyxo configurational family. Figure 7 also presents the spectrum of methyl β-D-riboside, the only member of the ribo family investigated. This spectrum, which shows a low-intensity negative band at 180 nm and a positive band below 165 nm, is also the result of compositionally weighted rotational strength contributions from both the C1 and 1C conformers.

Discussion

Difference Spectra. Following previous work we assume that CD bands are the net result of rotational strength created by pairwise interactions between groups in the molecule (the pairwise approximation).[17] Here we divide the molecule into the chromophoric functional groups HC(OH)<, $-CH_2OH$, and $-OCHOCH_3$. Under the pairwise approximation, the difference CD spectra of sugars which differ only at a single configurational center reveal the changes in the interactions involving the groups attached to this center with other groups in the molecule.

Difference spectra of homomorphic pairs of methyl aldopyranosides which differ only in the type of substituent attached to C-5 are given in Figure 8. These spectra reflect the contribution that the interactions involving an exocyclic hydroxymethyl group at the position make to the CD. A comparison of the glucoside, xyloside difference spectra shows that these interactions result in the addition of positive rotational strength to the CD spectrum irrespective of the configuration at the anomeric center. There are several similarities between these difference spectra and those of the corresponding aldopyranose pairs[2] which are surprising in view of the vast differences between the individual CD spectra. These similarities suggest that (1) the electronic, solvational, and conformational changes that occur upon the addition of a hydroxymethyl group to C-5 of a xylose or xyloside ring are similar, (2) the distribution of hydroxymethyl conformations must be essentially the same in both cases, (3) at least some of the lowest energy transitions must be the same in the pyranose and pyranoside sugars, (4) the pairwise approximation appears valid for homomorphic sugars having the same pyranoid ring conformation, and (5) the contribution that the C-5 hydroxymethyl group makes to the CD of the glucose and glucosides is only slightly dependent on the configuration and type of substituents

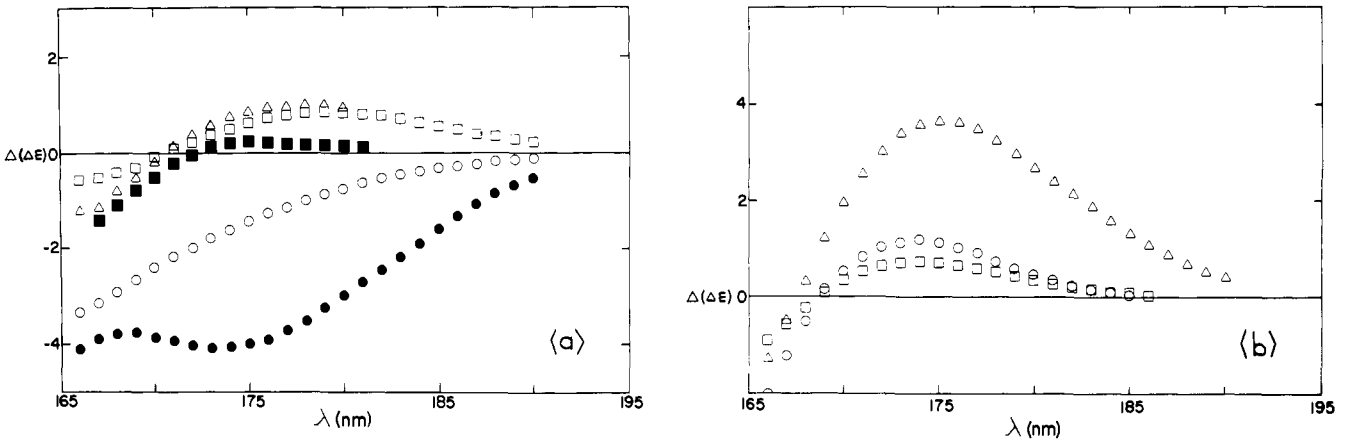

Figure 9. Difference CD spectra of methyl aldopyranoside epimers: (a) (○) α-D-galactoside minus α-D-glucoside, (●) β-D-galactoside minus β-D-glucoside, (□) α-D-mannoside minus α-D-glucoside, (△) 2-deoxy-α-D-glucoside minus α-D-glucoside, (■) β-L-arabinoside minus α-D-xyloside; (b) (△) α-D-galactoside minus β-D-galactoside, (□) α-D-xyloside minus β-D-xyloside, (○) α-D-glucoside minus β-D-glucoside.

attached to the anomeric C-1 carbon atom implying that only pairwise interactions with near neighbors are important here.

The α-D-galactoside minus β-L-arabinoside homomorphic difference spectrum shows that the introduction of the hydroxymethyl group to C-5 when the hydroxyl group at C-4 is oriented axially results in a negative contribution to the CD spectrum. The similarity between the α-D-xyloside and the β-L-arabinoside spectra (Figures 2 and 5) indicates that C-4 epimerization has only a small effect on the first two pentoside bands. Apparently these signs of these difference spectra in Figure 8 are associated with the rotamer distribution of the hydroxymethyl group which the C-4 configuration is shown to influence. Also, the hydroxymethyl group is probably acting as a perturber rather than a chromophore here. The CD difference spectrum for the α-D-mannoside, α-D-rhamnoside pair shows that a methyl group has the same effect as a hydroxymethyl in the long-wavelength region.

Figure 9a shows the difference CD spectra for five pairs of methyl aldopyranoside epimers. The two C-4 epimeric pairs give a negative difference spectrum which is similar in each case to the difference spectrum of the corresponding aldopyranose pair.[2] In particular, the difference spectra for the α and β anomers of these C-4 epimeric pairs show the same features which led us to postulate in paper 3 that there might be some difference in the rotameric distribution of the hydroxymethyl group between the anomeric forms of galactose. In any case, these observations suggest that the conversion of the characteristic pyranose hemiacetal grouping to the pyranoside methyl acetal grouping has essentially no effect on the conformation of the pyranoid ring, on the distribution of C-5 hydroxymethyl group conformation, or on some of the transitions responsible for the long-wavelength CD bands.

The changes in the CD that result from C-4 epimerization, without the presence of the hydroxymethyl group, can be seen in Figure 9a. β-L-Arabinoside minus α-D-xyloside spectrum shows that the epimerization, at least when the anomeric methoxyl group is oriented axially, produces a substantial change in the CD spectrum only below 175 nm.

The α-D-mannoside minus α-D-glucoside spectrum reflects the CD changes that occur upon C-2 epimerization. The changes are similar to those observed upon epimerization at the same locus in the ketopyranose sugars.[2] In each case the epimerization results in a positive contribution to the CD spectra at wavelengths longer than 170 nm. The last difference spectrum in Figure 9a shows the CD changes that occur upon a removal of the equatorial C-2 hydroxyl giving 2-deoxy-α-glucoside. Surprisingly, they are essentially identical with those that occur upon equatorial to axial epimerization of this hydroxyl group. One possible explanation is that an axially oriented C-2 hydroxyl group does not contribute to the CD of α-anomeric sugars in the C1 conformation.

The CD difference spectra of anomeric pairs of methyl aldopyranosides (C-1 epimers) are presented in Figure 9b where the CD spectra of the β anomer has been subtracted from that of the α anomer for D-galactoside, D-glucoside, and D-xyloside. In the long-wavelength region these positive differences are quite similar to those found for the corresponding aldopyranoses. We note that (1) the magnitude of the CD changes follows the same order in the two classes of monosaccharides, D-galacto greater than D-gluco greater than D-xylo; (2) in both classes the D-galacto difference is larger than and red shifted from the D-gluco and D-xylo difference spectra; and (3) in the long-wavelength region of the difference spectra even the magnitude of the analogous CD changes are similar. All these similarities indicate that for the most part the long-wavelength absorption bands of these two classes of monosaccharides are due to the same chromophore.

Similarities between the aldopyranoses and methyl aldopyranosides are summed up in Figure 10. For the β anomers the change from a hydroxy to a methoxy group on the anomeric carbon causes a negative CD change of moderate intensity beginning at 190 nm with a maximum about 170 nm. The changes for all three pairs are almost superimposable. In the case of the α anomers, the changes are almost superimposable for the D-xylo and D-gluco pairs but somewhat different for the D-galacto pair. All three are negative with intensity onset about 190 nm, but the -galacto difference spectrum is much less negative below 180 nm. This difference suggests that some type of conformational change occurs on going from α-D-galactose to α-D-galactoside which does not occur for the other two pairs of α anomers nor for the β-D-galacto pair.

Chromophores. One would like to be able to use CD spectra of sugars to assess their configuration and conformation. As a step in this direction it is important to know which of the many chromophores in the sugar molecule (hydroxyl, methoxyl, hydroxymethyl, hemiacetal, and acetal) are responsible for the various CD bands in the region investigated. If this can be sorted out, then one could use CD spectra to study the configuration and conformation of each group in the molecule. Listowsky et al.[11,12] suggested that the long-wavelength transition in pyranoid sugars is due to the ring oxygen atom. This was postulated because the first transition in tetrahydropyran occurs slightly to the red of the first transition in alcohols when these molecules are studied in the gas phase.[18-20] However, since it is generally agreed that these transitions are due to the excitation of nonbonding electrons on the oxygen atom, the transitions may well not occur at the same relative

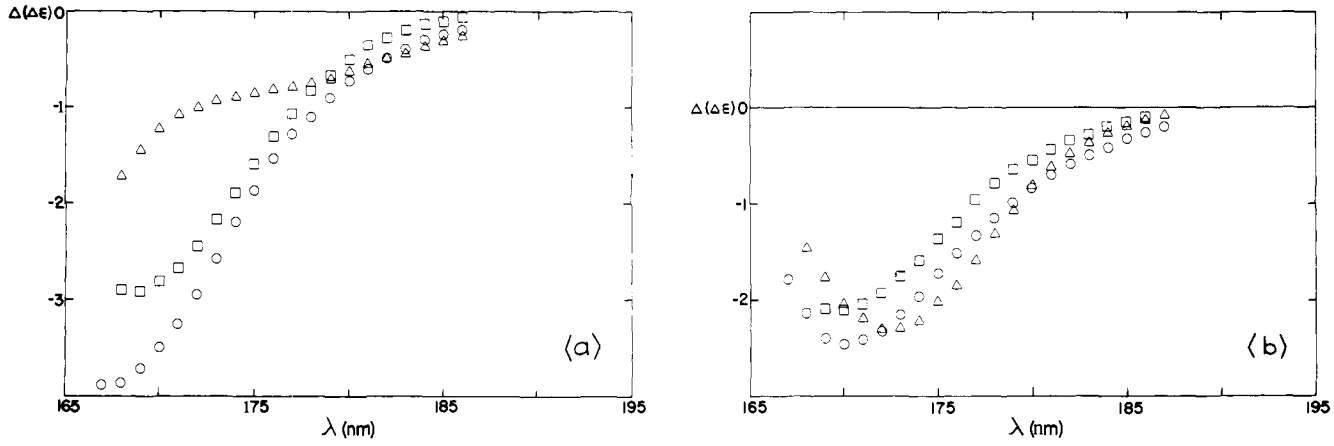

Figure 10. Difference CD spectra of methyl aldopyranosides and aldopyranoses: (a) α anomers (Δ) α-D-galactoside minus α-D-galactose, ($\square$) α-D-xyloside minus α-D-xylose, ($\circ$) α-D-glucoside minus α-D-glucose; (b) β anomers (Δ) β-D-galactoside minus β-D-galactose, ($\square$) β-D-xyloside minus β-D-xylose, ($\circ$) β-D-glucoside minus β-D-glucose. D-Galactose and D-glucose spectra have been red shifted 2nm before subtraction from respective pyranoside spectra to account for solvent difference. D-Xylose spectra have been red shifted 3nm before subtraction from the D-xyloside spectra to account for solvent and temperature difference.

energy in aqueous solution as they do in the gas phase. Interactions with hydrogen-bonding solvents are known to have a large effect on the energy of transitions due to nonbonding electrons. Here we see CD bands shifted far to the blue relative to CD bands for alcohols measured in hexane[21] and the gas phase.[22]

Previous work[2] has shown that the magnitude of these shifts may vary. For example, the outset of band intensity for α-D-galactose is 7.5 nm to the blue of the onset for β-D-galactose. However, as we shall see, it appears that the first transition is indeed due to the ring oxygen atom, but this transition exhibits large shifts in wavelength depending upon how the chromophore is shielded from the hydrogen bonding of its aqueous solvent environment.

The 12 methyl aldopyranosides studied give us enough information to tentatively assign CD bands observed in the vacuum ultraviolet to specific chromophores. The first point to note is that the sign of the second CD band (which occurs at 174 nm in the pyranosides in which it is clearly visible) and the third band (which peaks below 165 nm) is correlated with the configuration at the anomeric carbon. For instance, in the case of α- and β-D-xyloside (Figure 2) the α anomer exhibits a positive second band and a negative third band while the β anomer, which has the mirror image configuration around the anomeric carbon, exhibits opposite signs for these two bands. This arrangement is evident in the CD spectra of all the other methyl pyranosides measured except β-D-glucoside (Figure 3) and α-D-galactoside (Figure 4) where intensity from another CD band apparently overshadows the intensity of the 174-nm band. This suggests that the 174-nm band and at least part of the intensity of the band which peaks below 165 nm are due to the methoxy group.

In order for the methoxy group to give equal and opposite CD bands on anomerization, all its pairwise interactions with other groups in the sugar must be equal and change sign. While this is clearly not true for the molecule as a whole, it does turn out to be true for the groups near the methoxy chromophore. The methyl of the methoxy group is expected to be gauche to the anomeric hydrogen and the ring oxygen for both anomers.[23,24] Thus on anomerization the group gives equal and opposite pairwise interactions with the three other groups attached to the anomeric carbon as well as an equatorial hydroxyl attached to C-2.

Apparently hydroxyl chromophores, which hydrogen bond well with the aqueous solvent, contribute intensity below 175 nm with band maxima below 165 nm. This is clearly seen in a number of cases. The difference CD spectrum for α-D-mannoside, which has a hydroxymethyl group attached to C-5, and α-D-rhamnoside, which has only a methyl group attached to C-5 (Figure 8), has only short wavelength CD intensity. Figure 9 shows that changing a hydroxyl group at C-4 from equatorial to axial when there is no hydroxymethyl attached to C-5 (which might change its rotamer population and therefore affect the CD of the ring oxygen atom) causes a significant intensity change only at wavelengths shorter than 170 nm.

One might expect the anomeric hydroxyl in pyranoses to give rise to a short wavelength band which changes sign on change of configuration about C-1, in analogy to the methoxy group in methyl pyranosides. One expects the hydrogen of the anomeric hydroxyl to prefer the position gauche to the ring oxygen and anomeric hydrogen.[23,24] A complete change in the sign of the short wavelength band is not observed for any of the three anomeric pairs measured, however. Probably the bands due to the anomeric hydroxyl are bluer than the limits of solvent transmission or the intensity for this band is weak compared to the intensity contributed by the other hydroxyls in the monosaccharide. This last interpretation is consistent with CD spectra for the anomeric forms of D-xylose and D-glucose presented previously.[2] There we saw similar difference CD spectra for the two anomeric pairs with intensity onset of about 180 nm and a maximum ($\Delta\epsilon \sim 1.5$) at about 170 nm. The D-galactose anomers could not be compared because they apparently have different hydroxymethyl conformations.

As Listowsky and Englard[12] have pointed out in their pioneering work involving the CD spectra of methyl glycosides in the far-ultraviolet, the configuration about the anomeric carbon does not determine the sign of the first band in glycosides. Here the entire first band has been measured. Our results confirm their interpretation that this band is due to the ring oxygen and its sign is determined by the configuration of the groups about this chromophore. Certainly, assigning this band to the other ether chromophore is consistent with assigning the methoxy ether at lower energies and the observation that the effects of hydroxyls in difference spectra appear at higher energies.

The sign of the first band in pyranoses is also independent of the configuration about the anomeric carbon but is sensitive to the configuration of groups about the ring oxygen, particularly the conformation of the hydroxymethyl group at C-5.[2] This suggests that the first transition in simple monosaccharides is also due to the ring oxygen atom. This interpretation would mean that the first band is also present in the spectra of α- and β-D-xylose, although it is certainly not obvious. It is

suggested that the addition of a hydroxymethyl group to the C-5 carbon of the D-xylose ring shields the ring oxygen atom from the aqueous solvent thereby weakening or eliminating hydrogen bonding and shifting the band due to ring oxygen to the red. This interpretation is consistent with the idea that the sign of the first CD band in pyranoses is dependent on the rotamer population of the hydroxymethyl group. In the case of the pyranosides then, it must be the methoxy group which tends to shield the ring oxygen from the hydrogen bonding solvent, since methyl α- and β-D-xyloside as well as methyl β-L-arabinoside, which have no hydroxymethyl group, still display a long-wavelength CD band which is apparently due to the ring oxygen atom.

While the aldo- and ketopyranoses have at most one obvious band at wavelengths longer than 174 nm, the methyl pyranosides (see, for instance, D-xyloside, Figure 2) have two. The fact that the first two bands do not change between α-D-xyloside and β-L-arabinoside suggests that these bands are due to a chromophore which is not near the C-4 hydroxyl. Also, chromophoric nonbonding electrons on an ether are apparently more easily shielded from a hydrogen-bonding solvent than is the case for an alcohol chromophore. Thus, if the first band in methyl pyranosides is due to the ring oxygen, one expects the second band to be due to the methoxyl group. Actually, earlier work on the vacuum ultraviolet absorption spectra of model sugar compounds indicated that it might be best to treat acetal and hemiacetal groups as a single chromophore.[20] The results here indicate that one can treat the two oxygen atoms in these chromophores separately. Since the two oxygen atoms are only separated by a single carbon, some mixing undoubtedly takes place so that assigning bands simply to a ring ether oxygen or a methoxyl ether oxygen is probably something of an oversimplification.

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References and Notes

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Biosynthesis of Ophiocarpine. Introduction of a Stereospecific Label through Transannular Cyclization[1]

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Abstract: Results from ^{14}C labeling indicate that (−)-ophiocarpine (**8**) is biosynthesized in *Corydalis ophiocarpa* plants from scoulerine (**2**) via tetrahydroberberine. Nandinine (**9**) is only poorly incorporated into (−)-ophiocarpine and the biosynthetic sequence, **2** → isocorypalmine (**12**) → **10** → **8**, is suggested. Methods based upon a highly stereoselective transannular cyclization of the *trans*-dibenzazacine (**15**) and its 13-^3H derivative are shown to provide an efficient route for the synthesis of [13α-^3H]- and [13β-^3H]tetrahydroberberines (**30** and **31**) with high configurational purity. Incorporation experiments with [8,14-$^3H_2$]-, [13α-^3H]-, and [13β-^3H]tetrahydroberberines have established that the hydroxylation of (−)-tetrahydroberberine to (−)-ophiocarpine in *C. ophiocarpa* proceeds with retention of configuration involving the removal of the pro-*R* hydrogen atom from the C-13 position of tetrahydroberberine.

Studies of the biosynthesis of benzylisoquinoline alkaloids during the past 15 years have led to the elucidation of the major details of the steps involved in the construction of the skeletal framework of the principal classes of alkaloids of this family.[2] Despite the tremendous range in structural types presented by these alkaloids, their biosynthesis is linked by a common pathway from tyrosine to the simple benzylisoquinoline reticuline (**1**) which serves as the ubiquitous precursor of all members of this family so far studied. Second in importance to reticuline as a biosynthetic intermediate in this series are representatives of the protoberberine alkaloid series exemplified by scoulerine (**2**). Not only do these alkaloids constitute a large and important class of bases but certain members occupy a central position between reticuline and alkaloids of wide structural diversity representing the protopine (**3**), rhoeadine (**4**), benzophenanthridine (**5**) and phthalide isoquinoline (**6**) groups. In each case evidence supporting the intermediacy of the appropriate protoberberine base has been provided by tracer experiments.[3-6]

In addition to these alkaloid types, the spirobenzylisoquinolines such as ochotensine (**7**) are yet another class of alkaloid whose biosynthesis is thought to proceed via a proto-