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An Analysis of the Circular Dichroism Spectra of Uronic Acids*

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ABSTRACT: The circular dichroism spectra of a number of uronic acids with equatorial hydroxyl groups at C-4 were examined and compared with the spectra of uronic acids with axial hydroxyl groups at C-4. In aqueous solution, the derivatives of glucuronic and mannuronic acids exhibited negative circular dichroism bands near 234 mμ and positive bands with maxima near 206 mμ. Only a positive band near 210 mμ was obtained for galacturonic acid and its derivatives. For the glucuronic and mannuronic acid derivatives, it appeared that the true position of the band was obscured because of the overlap of the two bands of opposite sign. Thus, in nonaqueous solvents the negative band was more pronounced and was centered near 225–228 mμ. This increase in magnitude of the negative band was always accompanied by an attendant decrease in the magnitude of the positive band. Differences in the mode of solvation of two distinct structural species of these uronic acids in solution probably account for the presence of two bands.

The studies on mucopolysaccharides such as heparin (Stone, 1967) and chondroitin sulfates (Davidson, 1965; Stone, 1965; Eyring and Yang, 1968) are indicative of the usefulness of optical rotatory dispersion and circular dichroism measurements for the study of carbohydrate conformation (Beychok, 1968). Examination of the optical rotatory dispersion and circular dichroism spectra of monosaccharide constituents of known conformations may therefore be essential to facilitate the interpretation of results obtained with the polymers. In this connection features of the optical rotatory dispersion and circular dichroism spectra in the far ultraviolet (Pace *et al.*, 1964; Listowsky *et al.*, 1965, 1966; Englard *et al.*, 1966; Listowsky and Englard, 1968) have been correlated to various structural aspects of neutral sugars and their glycosides that do not absorb at wavelengths above 190 mμ. Also preferred alignments of the C-2 acetamido substituents in oligosaccharides obtained from milk and from blood group substances have been proposed on the basis of optical activity measurements (Beychok and Kabat, 1965; Lloyd *et al.*, 1967, 1968).

In a recent publication (Listowsky *et al.*, 1968) positive Cotton effects centered near 210 mμ and negative Cotton effects below 190 mμ were reported for a number of uronic acids. The rotatory contribution of the carboxyl group was isolated from the background rotations of the remainder of the molecules, and it was shown that the optical rotatory dispersion curves of uronic acids with equatorial hydroxyl groups at C-4 were characterized by small troughs near 240 mμ. Eyring and Yang (1968) in their study of chondroitin sulfate C also reported the presence of an optically active absorption band near 235 mμ for glucuronic acid.

In the present study, the circular dichroism spectra of selected uronic acid derivatives have been obtained, and attempts are made to relate the circular dichroism properties to specific structural features of these compounds. The effects of solvent environment are also explored in an effort to determine some of the characteristics of the electronic transitions for these compounds.

Experimental Section

Materials. Methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranosiduronic acid was a gift from Dr. G. O. Aspinall. The other uronic acid derivatives were optically

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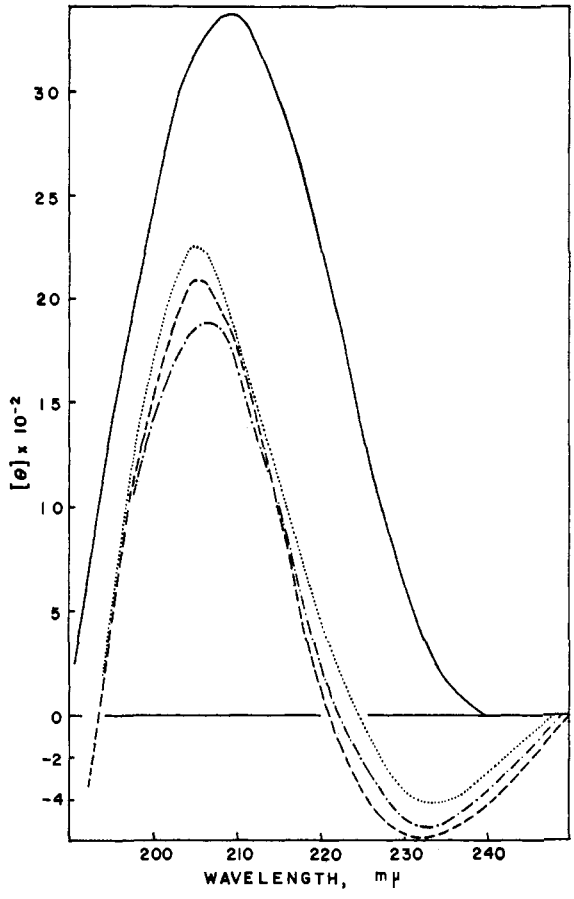

FIGURE 1: Circular dichroism spectra of glycosides of uronic acids. Methyl α-D-galactopyranosiduronic acid, ——; propyl β-D-glucopyranosiduronic acid, ...; methyl α-D-glucopyranosiduronic acid, – – –; and methyl α-D-mannopyranosiduronic acid, –·–·–. Uronic acid concentrations of 0.01–1.0% in aqueous solutions adjusted to pH 2.5–2.7 were employed.

pure materials that were obtained for an earlier study (Listowsky *et al.*, 1968). Examination of the uronic acid solutions by infrared spectroscopy indicated the absence of lactone forms. The aqueous solutions of the uronic acids were adjusted to pH 2.5–2.7 unless otherwise indicated.

Apparatus. Circular dichroism spectra were obtained using the 6001 circular dichroism accessory for the Cary Model 60 spectropolarimeter. This instrument records the angle of ellipticity, θ, in degrees, and molecular ellipticity, $[\theta]$ (deg cm²/dmole), was calculated using the relationship, $[\theta] = \theta M/10lC$, where M is the molecular weight, C is the concentration in grams per milliliter, and l is the path length in centimeters. Cylindrical quartz cells having path lengths of 0.005, 0.02, 0.1, 1.0, and 5.0 cm were employed. The shorter path-length cells were used mainly for concentration-dependent studies and for the experiments in dioxane. The circular dichroism measurements were all made at 27°, the temperature of the cell compartment.

Results

The circular dichroism spectra for the glycosides of

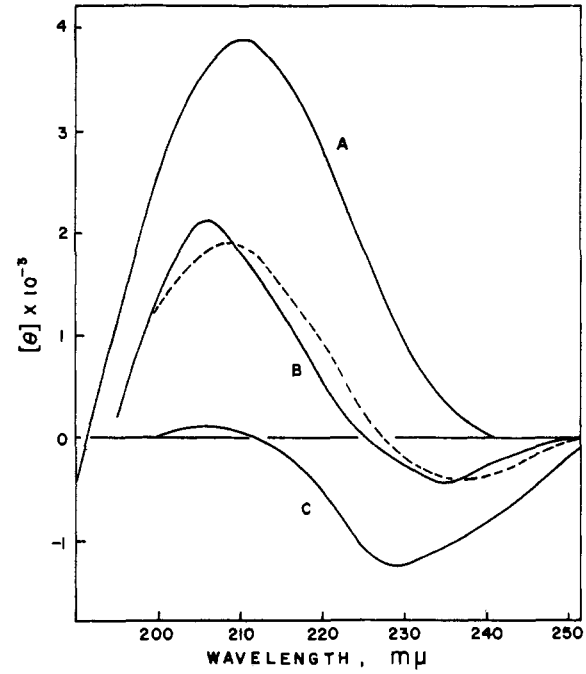

FIGURE 2: Circular dichroism spectra of galacturonic acid in water at pH 2.5 (curve A), glucuronic acid in water at pH 2.5 (curve B), and glucuronic acid in 95% dioxane solution (v/v) (curve C). The dotted line represents a calculated curve obtained from half the sum of curves A and C.

glucuronic and mannuronic acids feature positive ellipticity bands near 205 mμ and weak negative bands at 230–235 mμ (Figure 1). Methyl α-D-galactopyranosiduronic acid exhibits a more intense positive band at 210 mμ, but a negative band is not discernible. These circular dichroism results are all essentially independent of uronic acid concentration, and the presence of 8 M urea or gaunidinium chloride did not produce any changes in the spectra. The spectra for methyl α-D-mannopyranosiduronic acid and methyl α- and propyl β-D-glucopyranosiduronic acids are very similar to each other, indicating that the band intensities are unrelated to the configuration at C-2 or at the anomeric carbon atom.

The circular dichroism properties of glucuronic and galacturonic acid in aqueous solution (Figure 2) follow the same pattern as those of their glycosides. Thus, a negative band centered at 234 mμ is observed in the spectrum of glucuronic acid but not in the spectrum of galacturonic acid. In 95% dioxane the positive band for glucuronic acid is barely discernible, but the negative band is almost threefold that obtained in water. On the other hand, the shape of the circular dichroism spectrum of galacturonic acid is not substantially influenced by the presence of 95% dioxane as only a slight decrease in molecular ellipticity and shift in band position from 210 to 212 mμ are observed. A curve with two bands separated by 28 mμ that closely resembles the spectrum obtained for glucuronic acid in water can be constructed (dotted line, Figure 2) from the composite contributions of the positive band of galacturonic acid at 210 mμ (spectrum A, Figure 2) and the negative band for glucuronic acid in 95% dioxane at 228 mμ (spectrum C, Figure 2).

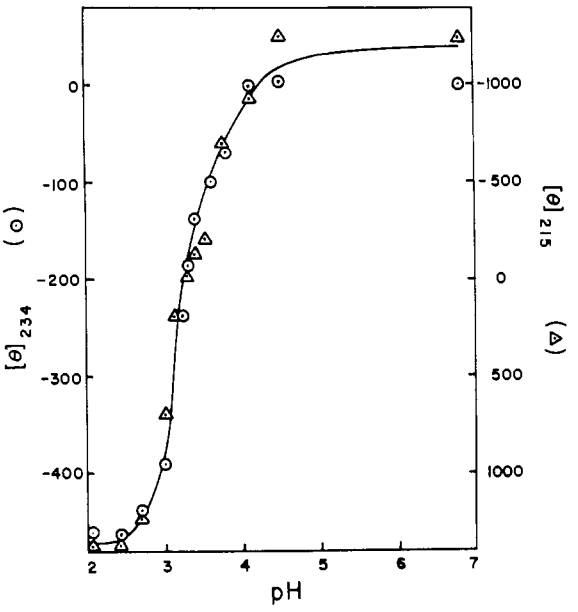

FIGURE 3: The effect of pH on the negative ellipticity band of glucuronic acid. The triangles represent the 215-mμ band, and the circles represent the 234-mμ band.

The magnitude of the positive bands are always greater for the uronic acids that do not display the negative band. Also, the molar ellipticities at 234 mμ for glucuronic acid and both of its glycosides are almost identical, indicating that the contribution of open-chain aldehydo forms or some nonspecific impurities are not appreciable. At neutral pH, the circular dichroism absorption bands for these compounds undergo shifts to shorter wavelengths, with positive bands of diminished intensity now located near 202 mμ and more intense negative bands at 215 mμ. The appearance of the 215-mμ band or disappearance of the 234-mμ band may be employed to measure the ionization of the carboxyl group of the uronic acids. A titration curve for glucuronic acid is shown in Figure 3, and the transition near pH 3.2 is consistent with the expected pK value for glucuronic acid (Haug and Larsen, 1961). Because of the presence of both the ionized and protonated forms, the circular dichroism curves obtained at pH 3.2–3.4 give the appearance of having a single broad negative band centered near 221 mμ.

To determine the influence of solvation and solvent polarity on the circular dichroism spectral characteristics of the uronic acids, methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranosiduronic acid was examined in view of its solubility in both hexane and aqueous media. In water, the circular dichroism curve of methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranosiduronic acid (Figure 4) resembles the spectra for glucuronic acid and its glycosides (Figures 1 and 2), with the negative band centered at 235 mμ and a positive band near 208 mμ. In hexane, however, the pattern is distinctly different, as only a single negative ellipticity band centered near 225 mμ is evident. The intensity of this negative band is independent of concentration (from 0.1 to 10 mg per ml) and it is much greater than the 235-mμ negative band observed

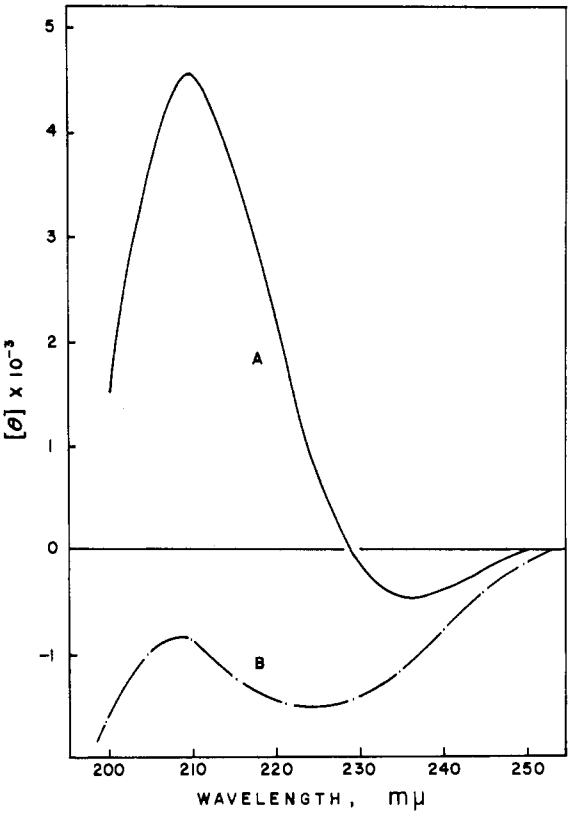

FIGURE 4: Circular dichroism spectra of methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranosiduronic acid in water at pH 2.5 (curve A) and in hexane (curve B).

in aqueous solution. In addition, the beginning of a second negative band below 200 mμ is discernible.

The effect of dioxane on the circular dichroism properties of glucuronic acid is shown in Figure 5. As the concentration of dioxane is increased, an increase in the intensity of the negative ellipticity band and attendant decrease in the positive ellipticity band are observed. Dioxane also induces a shift of the negative band to shorter wavelengths so that in 95% dioxane it is centered near 228 mμ.

Discussion

The circular dichroism spectra of uronic acids with axial substituents at C-4 (derivatives of galacturonic acid) can clearly be distinguished from the spectra of uronic acids with equatorial substituents at C-4 (derivatives of glucuronic and mannuronic acid). A negative circular dichroism absorption band centered near 234 mμ and a positive band near 206 mμ characterize the spectra of the latter compounds. From a cursory examination of the electronic absorption spectra of these compounds, only a single band near 210 mμ would have been anticipated. Indeed, for the galacturonic acid derivatives only a single positive circular dichroism band near 210 mμ is discernible, and it is improbable that a second transition at higher wavelengths is concealed by the end absorption of this band. The optically active absorption band near 234 mμ may be useful for the study and identification of these uronic acids in polysaccha-

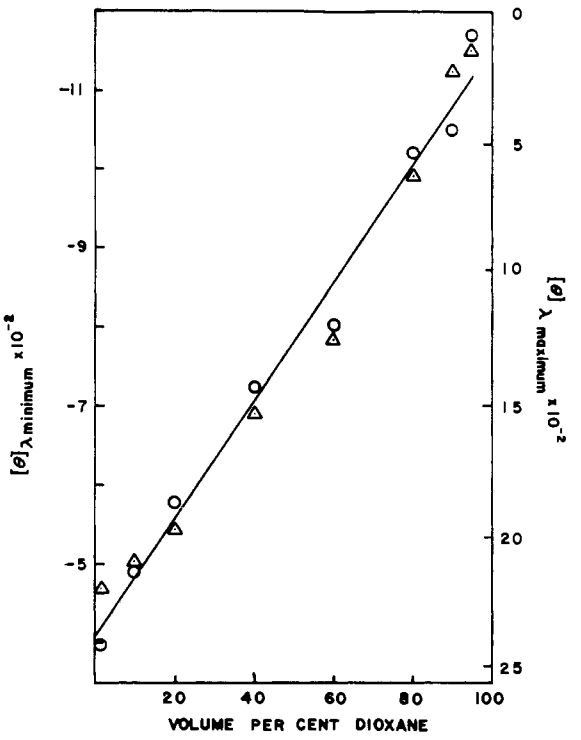

FIGURE 5: The effect of dioxane on the circular dichroism properties of glucuronic acid. $[\theta]_\lambda$ minimum and $[\theta]_\lambda$ maximum represent the molar ellipticity values of the negative band (circles) and the positive band (triangles), respectively. The spectral positions of these bands are dependent upon the dioxane concentration, and the indicated molar ellipticity values were obtained at the center of the peak or trough.

rides, since most of the other functional groups in these polymers absorb at shorter wavelengths.

There are a number of plausible explanations for the appearance of the additional band in the circular dichroism spectra of the derivatives of glucuronic and mannuronic acids in aqueous solution. These bands may arise from transitions that result from two different solvation energies of a single molecular species, or from two different conformations of the uronic acids in solution. It has been suggested that solvation equilibria account for the presence of two Cotton effects in the optical rotatory dispersion curves of isofenchone and epiisofenchone, since a conformational equilibrium was considered unlikely for these rigid molecules (Moscowitz *et al.*, 1963). For rigid diamide molecules, where conformational equilibria are also improbable, solvent-dependent changes in optical rotatory dispersion bands were ascribed to changes in energy levels that result in changes in degree of mixing of excited states (Schellman and Nielsen, 1967; Balasubramanian and Wetlaufer, 1967). For the less rigid uronic acid structures, both solvation and conformational equilibrium must be considered in the interpretation of the observations reported in the present study. Thus, the circular dichroism spectra of glucuronic acid in dioxane show (Figure 5) that an increase in magnitude of the negative band is always accompanied by a decrease in the positive band. These results are compatible with a change in the relative amounts of two predominant structural or solvated forms of the glucuronic acid as the concentration of nonaqueous solvent is increased. The single negative band observed for methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranosiduronic acid (Figure 4) implies that a single species predominates in hexane solution. The absence of an additional band at higher wavelengths for the derivatives of galacturonic acid in aqueous solution (Figures 1 and 2) would accordingly be compatible with the contribution of a single molecular form.

The differences in the circular dichroism properties of galacturonic acid as compared to glucuronic and mannuronic acid are undoubtedly due to inherent stereochemical factors. The preferred orientation of an hydroxymethyl group at C-5 in a pyranoid ring was previously shown to be dependent upon the configuration at C-4 (Listowsky *et al.*, 1965). The configuration at C-4 in the uronic acids may in an analogous manner influence the preferred alignment of the carboxyl chromophore, or affect the conformation of the entire ring. For example, a carboxyl group that is doubly hydrogen bonded to the ring oxygen and C-4 hydroxyl group has already been suggested to explain the rates of hydrolysis of glucuronic acid derivatives (Marsh, 1966). A hydrogen bond between the carboxyl group and axial hydroxyl group at C-4 in galacturonic acid, however, precludes the formation of a second hydrogen bond with the ring oxygen. In addition, the compounds with equatorial hydroxyl groups at C-4 should display a greater tendency for intermolecular interactions in solution, because an axial group could interfere with the stacking of the molecules. In this context, it is interesting to note that X-ray diffraction studies have revealed specific intermolecular interactions between glucuronic acid molecules in the solid state (Gurr, 1963; Furberg *et al.*, 1963). To our knowledge, such studies have not been reported for galacturonic acid. Inter- or intramolecular interactions of functional groups or similar interactions could result in the appearance of an additional circular dichroism band since an asymmetrically hydrogen-bonded system can induce $n-\pi^*$ transitions characterized by large circular dichroism bands. Solvation properties of such systems would undoubtedly differ from those lacking these interactions. Evidence for the existance of intermolecular interactions, however, could not be obtained in the present study.

Definitive band assignments cannot easily be made for optically active transitions near 235 mμ of uronic acids on the basis of available theoretical considerations.[1] It should be emphasized, however, that the observed location of the negative band may not actually define the transition wavelength. Since the two bands of

[1] Anand and Hargreaves (1967) have reported a negative circular dichroism band near 245 mμ for (*S*)-(+)-lactic acid that was ascribed to an $n-\pi^*$ transition. Weak ellipticity bands (from 8 to 50 deg cm² per dmole) above 230 mμ have also been observed for a number of other optically active carboxylic acids (I. Listowsky, manuscript in preparation). For these compounds the actual position of the longer wavelength band may be obscured by more intense bands at shorter wavelengths. The great majority of the optically active acids, however, exhibited only a single band near 210 mμ.

opposite sign overlap, it is conceivable that the shorter wavelength positive band influences the shape of the negative band and *vice versa*. It has been shown (Wellman *et al.*, 1965) that if two overlapping bands of opposite sign and having maxima separated by 1–20 mμ are superimposed, the resulting curve will give the appearance of having two bands separated by 28–32 mμ. The spectral separation of the two bands for the uronic acids is about 28 mμ (the positive band is centered near 206 mμ, the negative band near 234 mμ). Therefore, contrary to appearances, the longer wavelength transition for these uronic acids is probably centered well below 234 mμ in a spectral region entirely compatible with an n–π* transition which can be ascribed to the lowest energy absorption band for carboxylic acids (Sidman, 1958). Indeed, as the influence of the positive band is diminished in dioxane solution (Figure 5), the negative band shifts to shorter wavelengths. Also, in 100% hexane solution the isolated negative band is located at 225 mμ (Figure 4).

Hydrogen bonding to solvent lowers the energy of the ground state relative to the excited state of an n–π* transition resulting in a blue shift of the band (Kasha, 1950; McConnell, 1952). In hexane where no hydrogen bonding to solvent is possible, only the negative band is present in the spectrum for methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranosiduronic acid. It is therefore suggested that (1) both the negative and positive bands for the uronic acids originate from n–π* transitions, (2) the shorter wavelength positive band results from a species in which the carboxyl group is hydrogen bonded to the water molecules, and (3) the longer wavelength negative band results from a "nonsolvated" species.

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