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CHARACTERIZATION OF THE FAR ULTRAVIOLET OPTICALLY ACTIVE ABSORPTION BANDS OF SUGARS BY CIRCULAR DICHROISM

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In recent studies, the far ultraviolet optical rotatory dispersion (ORD) properties of a large number of sugars were related to known features of their configurations and conformations in solution (Englard et al., 1966; Listowsky et al., 1965, 1966). The present study is an initial report of the far ultraviolet circular dichroism (CD) measurements that have now been made on a select group of these sugars. The beginning portion of CD absorption curves are evident in the spectral region below 200 mμ and although the maxima for most simple sugars are expected to appear at wavelengths shorter than those accessible to current instrumentation, the sign of the first optically active absorption band is clearly discernible.

The CD curves for D-glucose and D-galactose shown in figure 1, are representative of the types of curves obtained for the other sugars in this study. In the spectral region above 200 mμ the CD absorption is negligible for both sugars, however below 200 mμ the band attains an appreciable magnitude, and becomes distinctly positive for glucose and negative for galactose. The ORD curve for D-galactose is characterized by an inflection point near 208 mμ, marking a change in direction of rotation from positive to negative (Listowsky et al., 1965; Pace et al., 1964). The shape of the ORD curve in this region is evidently related to the negative CD band of galactose shown in figure 1. The CD data verify our previous conclusion that, contrary to appearances, the inflection point at

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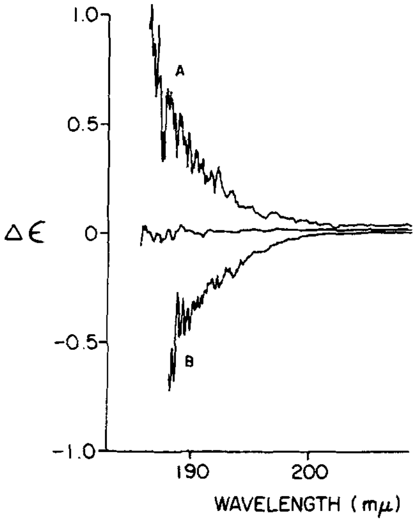

Figure 1. Circular dichroism spectra for D-glucose (curve A) and D-galactose (curve B) in water. The original spectra from the recorder charts of the Durrum-Jasco CD spectrophotometer are reproduced. The measurements were made on 0.11 M sugar solutions after mutarotational equilibrium was attained. A cell of 1 mm. optical path length was used. The CD absorption, $\Delta\epsilon$, was calculated from the relationship $\Delta\epsilon = \frac{\Delta D}{c \times d}$ where ΔD is the circular dichroic optical density, C is the concentration in gm. moles per liter, and d is the light path in cm.

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208 mμ is not the first peak of a positive Cotton effect, but instead is an approach to a negative Cotton effect trough at shorter wavelengths (Listowsky et al., 1965).

The CD curves for methyl α- and methyl β-D-glucopyranoside are both positive and similar in magnitude (Table I). The CD curves for methyl α- and methyl β-D-galactopyranoside and 6-deoxy-D-galactose are negative (Table I), but differ in magnitude. Although the sign of optical rotation is influenced to a significant extent by the configuration at the anomeric carbon atom (Listowsky et al., 1965), it is evident that the anomeric configuration does not determine the sign of the first CD band.

In the spectral region below 200 mμ, the first absorption bands of the ring oxygen of the pyranoid or furanoid forms are approached (Pickett et al., 1951) followed by the absorption of the hydroxyl groups at shorter wavelengths (Harrison et al., 1959). It has

TABLE I

Far Ultraviolet Circular Dichroic and Optical Rotatory Dispersion Properties of Selected Mono- and Disaccharides

Compound	$[\theta]_{190} \times 10^{-2}$ $\frac{\text{deg.cm}^2}{\text{decimole}}$ (1)	Rotational Direction at 190 mμ (2)
Methyl α-D-glucopyranoside	+ 2.0	(+)
Methyl β-D-glucopyranoside	+ 2.0	Level
Methyl α-D-galactopyranoside	- 0.7	(+) (3)
Methyl β-D-galactopyranoside	- 6.6	(-)
α-D-glucopyranosyl α-D-glucopyranoside	+ 3.3	(+)
4-O-α-D-glucopyranosyl-D-glucose	+ 0.7	(+)
4-O-β-D-glucopyranosyl-D-glucose	+ 2.6	(+)
4-O-β-D-galactopyranosyl-D-glucose	- 7.2	(-)
4-O-β-D-galactopyranosyl-D-fructose	- 7.0	(-)
6-Deoxy-D-galactose	- 5.2	(-)
D-Mannose	+12.0	(+)
Methyl α-D-mannopyranoside	+ 5.6	(+)
D-Talose	+ 0.9	(-) (3)

(1) Molar ellipticities ($[\theta]_\lambda$) were calculated from the relationship $[\theta]_\lambda \approx 2.303 \times \frac{4500}{\pi} \times \Delta\epsilon$

(2) Rotational directions obtained from data previously reported (Listowsky *et al.*, 1965; Englard *et al.*, 1966).

(3) Because of the overriding rotatory contribution of the α-anomeric form, the ORD curve for methyl α-D-galactopyranoside continues in the positive direction below 200 mμ; nonetheless the slope is much shallower than the slope of the curve for methyl α-D-glucopyranoside. The ORD curve for D-talose exhibits an inflection point at 208 mμ and change in direction of rotation (from positive to negative), due to the predominance of the β-anomeric form of this sugar at equilibrium. In general, the ORD data express the contributions of all of the asymmetric centers, whereas the CD data are more limited, therefore the sign of the CD band and the direction of rotation do not always correspond to each other.

therefore been postulated that the stereochemical alignment of groups about the ring oxygen determines the characteristics of the first optically active absorption band (Listowsky *et al.*, 1965). The influence of the configuration at C-4 on the freely rotating hydroxymethyl group at C-5 was cited as a major factor determining the ORD properties in the far ultraviolet region. In addition, an axial hydroxyl group at C-4 introduces a new axis of polarizability difference in the molecule because of the dissimilarity of the axial substituents at C-4 and C-2 (OH and H, respectively). The opposite signs of the CD bands for the sugars in the D-galactose series as compared to those for the glucose derivatives can be attributed to these factors.

The CD data for the disaccharides shown in Table I, agree with the general proposal that, for sugars in the C1 conformation, the presence of an axial hydroxyl group at C-4 determines a negative CD band, and an equatorial hydroxyl group at C-4 gives a positive band. Similarly, sugars containing axial hydroxyl groups at C-2 should exhibit positive curves. Thus, D-mannose and methyl α-D-mannopyranoside (Table I) exhibit positive CD bands of greater intensity than those of the corresponding glucose derivatives. Furthermore, D-talose, containing C-2 and C-4 axial substituents which contribute in opposite directions, exhibits a very weak positive CD curve.

Detailed analysis of the CD properties of sugars may provide empirical guidelines for study of specific aspects of sugar configuration and conformation, and to define the nature of the optically active transition(s) responsible for the observed CD band below 200 mμ. The restrictions in an ORD study resulting from combinations of the contributions of various asymmetric centers and contributions of various optically active transitions are not inherent in a CD study. It may therefore be possible to isolate and accurately assess the contributions of individual stereochemical alignments of atoms or groups in the sugar molecule by the analysis to the CD spectrum below 200 mμ.

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REFERENCES

Englard, S., Avigad, G., and Listowsky, I., Carbohydrate Res., 2, 380 (1966).
Harrison, A.J., Cederholm, B.J., and Terwilliger, M.A., J. Chem. Phys., 30, 355 (1959).
Listowsky, I., Englard, S., and Avigad, G., Carbohydrate Res., 2, 261 (1966).
Listowsky, I., Avigad, G., and Englard, S., J. Am. Chem. Soc., 87, 1765 (1965).
Pace, N., Tanford, C., and Davidson, E.A., J. Am. Chem. Soc., 86, 3160 (1964).
Pickett, L.W., Hoeflich, N.J., and Liu, T.C., J. Am. Chem. Soc., 73, 4865 (1951).