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A SYMMETRY RULE FOR THE CIRCULAR DICHROISM OF REDUCING SUGARS, AND THE PROPORTION OF CARBONYL FORMS IN AQUEOUS SOLUTIONS THEREOF*

L. DOUGLAS HAYWARD
Department of Chemistry, University of British Columbia, Vancouver, British Columbia V6T 1W5 (Canada)

AND STEPHEN J. ANGYAL
School of Chemistry, University of New South Wales, Kensington, New South Wales 2033 (Australia)

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ABSTRACT

From the circular dichroism spectra of 23 aldoses and 10 ketoses in aqueous solution, a symmetry rule was derived relating the sign of the $n-\pi^*$ band of the carbonyl form to the absolute configuration of the sugar. The sign is positive when the configuration of the adjacent chiral center is (*S*), and it is negative when the configuration is (*R*). The rule also holds for *aldehydo-* and *keto*-sugar peracetates, and for γ-lactones substituted with oxygen-containing groups.

The magnitude of a mean, molar, dichroic-extinction coefficient for the pure carbonyl forms of aldoses and ketoses was estimated from published c.d. data on the *aldehydo-* and *keto*-sugar peracetates and related compounds, which permitted estimation of the proportion of the carbonyl form in the equilibrium mixture for each sugar. D-Glucose had the smallest carbonyl concentration, 0.002% at 20°, in agreement with an earlier, polarographic value. The relationship between carbonyl content and sugar structure and configuration is discussed.

INTRODUCTION

It was reported more than 40 years ago[1,2] that fully acetylated *aldehydo*-sugars exhibit circular dichroism (c.d.) bands at about 290 nm, the frequency of the $n-\pi^*$ transition of the carbonyl chromophore. The failure to observe this band in the o.r.d.[3] and earlier c.d.[4–6] spectra of the unsubstituted aldoses in aqueous solution has generally been taken as evidence that the proportion of the aldehydo form is very small[7]. In the isotropic absorption spectra the $n-\pi^*$ band, if present, is masked[5] by the tail of a moderately strong band near 210 nm.

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*The Circular Dichroism of Reducing Sugars. Part I.

Recent work has shown the presence of a weak, carbonyl, c.d. band in aqueous solutions of D-glyceraldehyde[8], aldopentoses[9], and ketohexoses[4,5]. We now report similar c.d. bands in the spectra of the aldohexoses and some of their derivatives, recorded with a more sensitive circular dichrometer, and the c.d. spectra are then used as a basis for estimating the relative proportions of the carbonyl forms of the reducing sugars in aqueous solution.

RESULTS AND DISCUSSION

The correlation of the sign of the $n-\pi^*$, circular dichroism band with absolute configuration, first observed for the pentoses[9], has now been extended to all of the reducing sugars listed in Table I and may be expressed as a *symmetry rule*. *The sign of the $n-\pi^*$ circular dichroism band of a reducing sugar is positive when the configuration at the adjacent chiral center is* (S), *and it is negative when the configuration is* (R).

The rule also holds for the *aldehydo-* and *keto-*sugar peracetates, as reported by Lowry and Wolfrom and their coworkers[1,2], and for the 2-alkoxy-γ-lactones. Thus, the (*R*) isomer of pantolactone (2,4-dihydroxy-3,3-dimethylbutano-γ-lactone) gave $\Delta\varepsilon$ $-1.56\ \mathrm{M}^{-1}.\mathrm{cm}^{-1}$ at 225 nm in acetonitrile solution[10], and the same sign correlation was earlier found to hold for the aldono-γ-lactones[11,12] and their derivatives[13]. For the γ-lactones, Meguro and co-workers[13] were able to show that the sign-determining effect of the *alpha* substituent outweighs that of the ring geometry and also any effects of other ring substituents. If the 5-membered, lactone ring is regarded as planar, the sign of the $n-\pi^*$ band is determined by application to these compounds of the octant rule originally developed for substituted cyclohexanones[14], as well as the more elaborate, sector rule developed from the octant rule by Klyne and his colleagues[15] for lactones substituted with alkyl groups and cycloalkane rings.

Calculation of the percentage concentration of the carbonyl form of a sugar in aqueous solution from the observed c.d. spectrum required evaluation of the molar, dichroic-extinction coefficient for the pure carbonyl form, $\Delta\varepsilon^{\mathrm{CO}}$, at the band maximum. Until now, there had been practically no information on the proportion of the aldehydo form of aldoses in equilibrium in aqueous solution, except that it was too low to be detected by u.v. or n.m.r. spectroscopy. The only quantitative figure was that of 0.0026% for D-glucose at 25°, determined by polarography[16]. (Earlier, much higher, polarographic values[17] were shown to have been calculated on the basis of a wrong assumption[16]). However, solutions of ketoses contain higher proportions of the keto forms; the absorption thereof is clearly visible in u.v. spectra of aqueous solutions, but quantitative evaluation is difficult because the peak constitutes only a shoulder on the strong band near 210 nm.

In the absence of a direct method for determining $\Delta\varepsilon^{\mathrm{CO}}$ for each sugar, a mean, absolute value for aldoses and ketoses was estimated from the following data.(*a*)$\Delta\varepsilon^{\mathrm{CO}}$ values for (*S*)-3-methyl-2-pentanone and (*S*)-4-methyl-2-hexanone in methanol, and for (*S*)-2-methylbutanal and (*S*)-3-methylpentanal in heptane, were calculated from the o.r.d. curves[17–19] with the relationship[20] $\Delta\varepsilon = A/40.28$ as +0.2, −0.2, +0.2, and −0.3, respectively. (*b*) $\Delta\varepsilon^{\mathrm{CO}}$ values in chloroform solution for the peracetates of *aldehydo*-L-arabinose, -D-galactose, -D-glucose, 6-deoxy-*aldehydo*-L-galactose, and *keto*-D-fructose were −1.80, −2.25, −1.00, +2.15, and +1.00, respectively[1,2]. (*c*) Direct comparison of $\Delta\varepsilon^{\mathrm{CO}}$ values for hydroxybicyclo[2.2.1]heptanones with those for the corresponding *O*-acetylated compounds was complicated by the occurrence of bisignate $n-\pi^*$, c.d. bands[21,22]. The $\Delta\varepsilon^{\mathrm{CO}}$ values from the monosignate spectra of (1*R*,2*S*,4*S*)-2-acetoxy-1,5,5-trimethylbicyclo[2.2.1]3-heptanone in methanol and in cyclohexane were −0.9 and −1.2, respectively, and, from the monosignate spectrum of the corresponding alcohol in cyclohexane, was[21] +0.4. (*d*) The ratios of the total $n-\pi^*$, c.d.-band areas in methanol to those in hydrocarbon solvents, for both mono- and bi-signate spectra of the hydroxy- and acetoxy-bicyclo[2.2.1]heptanones with 1-, 2-, and 3-bond separations of the oxy and carbonyl groups[21,22], confirmed the expected increase[23] (15–35%) in $\Delta\varepsilon^{\mathrm{CO}}$ with increasing solvent-polarity. So far, no $\Delta\varepsilon^{\mathrm{CO}}$ values for hydroxyketones in aqueous solution appear to have been recorded, but, judging from the solvent effect on $\Delta\varepsilon^{\mathrm{CO}}$ of D-pantolactone[24], they could be expected to be similar to those obtained in alcohol, chloroform, and acetonitrile solutions. (*e*) In the hydroxy-5*β*-androstan-11-ones, *O*-acetylation of the 3*α*-hydroxyl group at 5-bond separation from the carbonyl group caused an increase in $\Delta\varepsilon^{\mathrm{CO}}$ from +0.25 to +0.36, and the appearance of a weak band at 325 nm in 1,4-dioxane solution[25]. Acetylation of hydroxyl groups at 8- and 9-bond separations in steroid ketones[25] caused only negligible changes in $\Delta\varepsilon^{\mathrm{CO}}$ for both endocyclic and exocyclic ketone groups.

On the foregoing evidence, the absolute magnitudes of $\Delta\varepsilon^{\mathrm{CO}}$ for the acyclic aldoses and ketoses in aqueous solution would appear to be similar, with a probable, mean value near unity. On this assumption and the relationship giving % of carbonyl form $= \Delta\varepsilon \times 100/\Delta\varepsilon^{\mathrm{CO}}$, the approximate proportion of the carbonyl form of each sugar was estimated as shown in the last column of Table I The value thus obtained for D-glucose, *viz*., 0.002%, was in satisfactory agreement with the earlier, polarographic value[16]. Direct evaluation of $\Delta\varepsilon^{\mathrm{CO}}$ for reducing sugars from the combined c.d. and n.m.r. spectra[26] is feasible.

The variation of $\Delta\varepsilon$ with temperature within families of diastereomers (see Figs. 1 to 4) indicated that the carbonyl contents of the aqueous solutions of the sugars generally increase with increasing temperature In most cases, the increase is about fourfold on raising the temperature from 20 to 60°, a smaller increase occurs with solutions of those sugars that contain considerable proportions of furanoses in equilibrium, presumably because the furanose content also increases with increasing temperature.

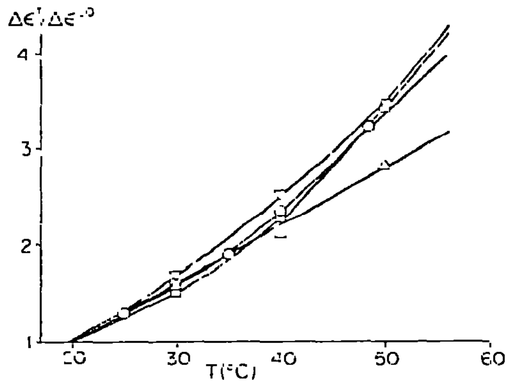
The figures in Table I show that the proportion of the aldehydo form in the solutions of aldohexoses varies by at least a factor of 3. The variations in these equilibria are due mainly to the different free-energies of the pyranose forms of the various sugars. These have been estimated by a simple method[27], and the present results are in accordance with these estimates. Thus, D-glucose, which has the most stable pyranose forms, shows the lowest carbonyl content of all sugars examined; D-idose, which has the least stable, pyranose forms, shows a high carbonyl content. It is noteworthy that D-idose, in contrast to the common aldohexoses, gives a positive

TABLE I

CIRCULAR DICHROISM AND ESTIMATED PROPORTION OF CARBONYL FORMS OF REDUCING SUGARS IN AQUEOUS SOLUTION AT 20°

Sugar	*Δε × 10³*	*λ (nm)*	*Alpha-carbon configuration*	*Estimated carbonyl*[a] (%)
D-Arabinose	+0.333	288	*S*	0.03
L-Lyxose	−0.255	290	*R*	0.03
D-Ribose	−0.469	285	*R*	0.05
D-Xylose	−0.156	293	*R*	0.02
D-Allose	−0.109	283	*R*	0.01
D-Altrose	+0.360	285	*S*	0.04
D-Galactose	−0.170	287	*R*	0.02
D-Glucose	−0.0222	285	*R*	0.002[b]
D-Idose	+1.82	288	*S*	0.2
D-Mannose	+0.0535	292	*S*	0.005
D-Talose	+0.336	285	*S*	0.03
D-*glycero*-D-*gulo*-Heptose	−0.195	290	*R*	0.02
D-*glycero*-D-*galacto*-Heptose	−0.138	286	*R*	0.01
D-*glycero*-L-*galacto*-Heptose	+0.206	286	*S*	0.02
5-Thio-D-glucose	−0.05	295	*R*	0.005
5,6-Di-*O*-methyl-D-glucose	−9.57[c]	289	*R*	1
3,6-Anhydro-D-glucose	−0.48	275	*R*	0.05
D-*threo*-2-Pentulose	+76.1	272	*S*	8
D-Fructose	+6.72[d]	273	*S*	0.7
D-Psicose	−3.30	265	*R*	0.3
L-Sorbose	+2.16[e]	273	*S*	0.2
D-Tagatose	+5.7	280	*S*	0.6
D-*altro*-3-Heptulose[f]	−27.4[c]	279	*R*, *R*	3
Turanose	+10.0[g]	283	*S*	1
1-Deoxy-D-fructose	+138	274	*S*	14
1-Deoxy-L-sorbose	+52.1	275	*S*	5
1-Deoxy-L-psicose	+104	271	*S*	10
2-Deoxy-D-*arabino*-hexose	−0.0776	288	*R*[h]	0.008
2-Deoxy-D-*lyxo*-hexose	−0.285	290	*R*[h]	0.03
6-Deoxy-L-galactose	+0.0111[i]	290[i]	*S*	0.007
6-Deoxy-D-glucose	−0.0182	297	*R*	0.002
6-Deoxy-L-mannose	−0.055[i]	290[i]	*R*	0.006
2,6-Dideoxy-D-*ribo*-hexose	+0.28	290	*S*[h]	0.03

[a] Based on an estimated, maximum, molar, dichroic-extinction coefficient of unity for the pure carbonyl form (see text). [b] Compare the polarographic value 0.0026% at 25° (ref. 16). [c] At 25°. [d] Previously reported as +10 (ref. 4) and +8.93 (ref. 5) at an unspecified temperature. [e] Previously reported as −3.73 for the D isomer (ref. 4). [f] "Coriose" (ref. 35). [g] Previously reported as +17° at an unspecified temperature (ref. 4). [h] *Beta*-carbon configuration. [i] At 27–30°.



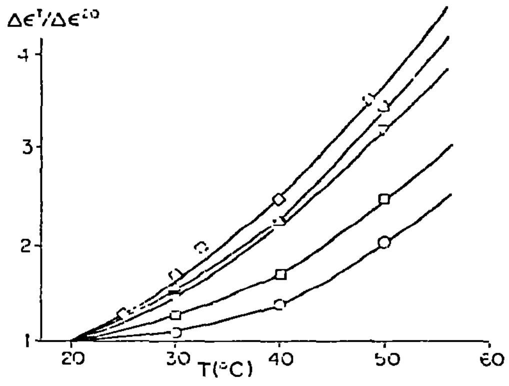
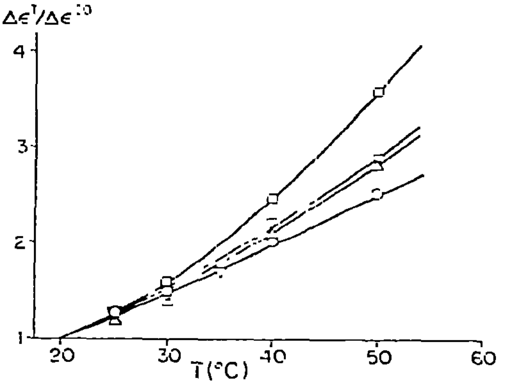


Fig. 1. Temperature dependence of the carbonyl circular dichroism of aldopentoses in aqueous solution (D-Arabinose □, L-lyxose ○, D-ribose ⊥, and D-xylose ⊤.)

Fig 2 Temperature dependence of the carbonyl circular dichroism of aldohexoses in aqueous solution. (D-Altrose □. D galactose ⊥, D-glucose ⊤, D-mannose ◊, and D-talose ⊃)



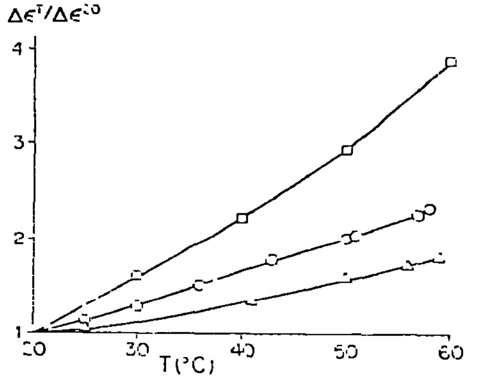


Fig 3. Temperature dependence of the carbonyl circular dichroism of ketohexoses in aqueous solution. (D-Fructose ○, D-psicose ⊤, L-sorbose □, and D-tagatose △.)

Fig. 4. Temperature dependence of the carbonyl circular dichroism of 1-deoxy-2-hexuloses in aqueous solution. (1-Deoxy-D-fructose ○, 1-deoxy-L-psicose ⊥, 1-deoxy-L-sorbose □)

Schiff reaction for aldehydes[28]. To a lesser extent (because the free-energy differences are smaller), the equilibrium is also affected by the relative stabilities of the acyclic forms[29]. The free-energies of the acyclic forms have not been estimated, but it is known that the most stable ones are those which are in the zigzag conformation. When 1,3-parallel interactions force the chain into a sickle form, *gauche* C–C interactions are present, and the free energy is higher. Mannose and galactose have the most favored conformations in the acyclic form: allose has the least favored (two *gauche* C–C interactions) and, hence, its aldehydo content is low, although the pyranose forms are less favored than those of galactose.

Because D-gulose has not yet been obtained crystalline, and is, therefore, not readily isolated in the pure state, the homomorphous D-*glycero*-D-*gulo*-heptose was investigated instead. This seems to be justified by the comparison of the data on D-galactose with those of the two *galacto*-heptoses; addition of an extra carbon atom outside the ring is seen not to alter the proportion of the carbonyl form. It is interesting that, of the two homomorphous heptoses, D-*glycero*-D-*galacto*-heptose shows the higher aldehyde content. Its acyclic form has no *gauche* C–C interaction, whereas that of D-*glycero*-L-*galacto*-heptose has one.

5,6-Di-*O*-methyl-D-glucose, which gives a positive Schiff test, can afford no pyranose forms; hence, in solution, the aldehydo form has to compete only with the (much less stable) furanose forms, and occurs in a much higher proportion than in aqueous solutions of D-glucose. If the reasonable assumption is made that the ratio of aldehydo to furanose forms is the same in solutions of 5,6-di-*O*-methyl-D-glucose as in those of D-glucose, then the latter contains about 0.3% of furanose forms in equilibrium. Because this proportion is too small to be observed by n.m.r. spectroscopy, it has not been previously estimated.

The pyranose forms of 3,6-anhydro-D-glucose are highly strained[7]; hence, here again, the aldehydo form has to compete only with the furanose forms, and so it occurs in a higher proportion than in solutions of D-glucose. Fischer and Zach[30] recorded that this compound gives a red color with the Schiff reagent. Haworth *et al.*[31] assumed that, in solution, the compound is mainly in the furanose forms, accompanied by a small proportion of the aldehydo form. The n.m.r. spectrum in aqueous solution shows only the two furanose forms, in approximately equal amounts: αH-1 at δ 5.46 with $J_{1,2}$ 4 Hz and βH-1 at δ 5.43 with $J_{1,2} \sim 1$ Hz; there is too little of the aldehydo form (see Table I) for it to be detected in the spectrum. In contrast to 5,6-di-*O*-methyl-D-glucose, the aldehydo form is not strain-free, because it contains a five-membered ring bearing three, consecutive *cis*-substituents; hence, an aqueous solution of 3,6-anhydro-D-glucose contains much less aldehydo form at equilibrium than does that of 5,6-di-*O*-methyl-D-glucose.

Among the aldopentoses, D-xylose, having the most stable pyranose forms, shows the lowest, and D-ribose, having the least stable pyranose forms, the highest, proportion of carbonyl form. Aqueous solutions of all the aldopentoses contain more aldehyde at equilibrium than do those of the homomorphous aldohexoses. As the solutions also contain a higher proportion of furanose forms than those of the aldohexoses[32], it appears that their pyranose forms are less stable. Apparently, ring closure through a primary hydroxyl group yields a less stable acetal than one through a secondary hydroxyl group. [A recent example is the behavior of dihydrostreptose, 5-deoxy-3-*C*-(hydroxymethyl)-L-lyxose, which appears to form glycosides by ring closure through the secondary OH-4, rather than the primary OH-3^1 group[33]]. D-Erythrose, which can only form furanose forms (and those through a primary hydroxyl group), would be expected to contain a much higher proportion of the acyclic form at equilibrium.

The solutions of the ketoses are seen to contain a higher proportion of carbonyl forms than those of the aldoses. L-Sorbose (L-*xylo*-hexulose), having the most stable pyranose forms[34], contains the least of the acyclic form. The behavior of D-psicose (D-*ribo*-hexulose) is somewhat puzzling; its pyranose forms have *syn*-axial hydroxyl groups, and hence a larger proportion of the keto form would have been expected. D-*threo*-2-Pentulose, which can only form furanoses, and those only through the primary hydroxyl group on C-5, shows a very high ketone content. D-*altro*-3-Heptulose[35] has a higher ketone content than the homomorphous D-psicose, because its side-chain is bulkier.

The aldehydo forms of sugars are extensively hydrated in aqueous solution[36,37]. It is, therefore, to be expected that the aldehydo forms herein reported are accompanied in equilibrium by 10–20 times larger amounts of the aldehydrol form; no method is yet known for its quantitative determination in the equilibrium mixture. The keto forms of the ketoses appear to form no substantial amounts of hydrates[38].

EXPERIMENTAL

The c.d. spectra were recorded in Vancouver with a JASCO J20 circular dichrometer fitted with a thermostatted sample-compartment maintained within ±0.1° by an external, circulating bath. Equilibrated solutions (0.2 to 1M) were prepared in distilled water (redistilled from potassium permanganate) and the pH of the sugar solutions lay in the range 5.2 to 7.0. Specific rotations were measured with a PE-141 automatic polarimeter, for solutions in thermostatted sample-tubes.

The carbonyl content of aqueous solutions of D-fructose did not appear to change during mutarotation. The c.d. band was fully developed in the first spectral scan, recorded 3 min after contact between the crystalline sugar and water at 25.0°, and remained constant over the next 96 h while the solution (pH 6.30) mutarotated from −105° toward the equilibrium value of −92° during the first hour. The c.d. spectrum of the equilibrated solution was unchanged on purging with oxygen, and after storage for several days in laboratory light.

The $\Delta\varepsilon$ values previously reported for D-glyceraldehyde and D-erythrose[8,9] are probably too large.

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