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Circular Dichroism and the Conformation of Sugars Having Vicinal Diacylamino Substituents

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Abstract: The circular dichroism spectra of carbohydrates vicinally substituted by acetamido groups differ fundamentally from those of the common 2-acetamido-2-deoxy sugars as a result of mutual coupling effects. The calculations presented in this paper follow a method due to Schellman and co-workers which has been widely used in peptide CD calculations and which treats both the exciton coupling and static field effects in a consistent manner. The calculations predict, in agreement with experiment, that the 189-nm amide $\pi-\pi^*$ transition is split into two oppositely signed CD bands of approximately equal magnitude near 180 and 200 nm. A small $n-\pi^*$ band is predicted near 210 nm. The sign and magnitude of the bands depend on the dihedral angles defining the orientation of the amides with respect to the hexapyranose ring. It is shown that the calculations yield two independent parameters which may be compared with experiment: the product of the splitting with the rotational strength for the exciton bands and the total rotational strength for the $n-\pi^*$ band. Comparison of the calculated results with the experimental spectra of 2,3-diacetamidoglucose and of the glycopeptide linkage compound, 2-acetamido-1-*N*(4-*l*-aspartyl)-2-deoxy-β-D-glucopyranosylamine, leads to the conclusion that both amide residues of the latter compound are oriented such that the relationship between the amide proton and the pyranose CH proton is trans while in the former the relationship is cis.

I. Introduction

2-Deoxy-2-acetamido sugars are common constituents of the complex oligosaccharide chains which are covalently attached to glycoproteins and glycolipids. Containing from 2 to 15 sugar units, these chains exhibit highly varied sequence and linkage often including branched structures. Although the biosynthetic pathway leading to these oligomers is exceedingly complex, little is known of their biological function. Even less is known of their three-dimensional conformation and of the forces governing their interactions with immunoglobulins, lectins, and hormones. The amide chromophore of the acetamido sugars serves as a convenient ultraviolet optical probe for conformational studies. The system whose CD[12] is treated in this work serves as a model for the region of covalent attachment of the sugar chain to the peptide chain of the "serum-type" glycoproteins.

The CD of 2-acetamido-2-deoxyhexoses has been the subject of both theoretical and experimental study. These carbohydrates, which have a single amide function, show a CD band near 209 nm which is assigned to the electrically forbidden amide $n-\pi^*$ transition. The rotational strength arises mainly from a one-electron mechanism in which the asymmetric field of the atoms near the amide induces optical activity in the chromophore.[1] Since the major electrostatic perturber is the hydroxyl at C-3, changes in the solvent which modify the orientation of this group have a striking solvent effect on the CD spectrum.[2] In addition to the band near 209 nm, methyl glycosides of compounds such as GlcNAc and GalNAc show CD bands in the 185–192-nm region which are due to the $\pi-\pi^*$ transition of the amide chromophore which has a strong absorption band at 189 nm. The magnitude and position of these shorter wavelength bands depends in a regular way on the configuration of the glycosidic linkage.[3] Similar results are found for oligosaccharides composed of *N*-acetylamino sugars such as the chitin oligosaccharides.[3,4]

The CD spectra of sugars vicinally substituted with two amide residues contrast sharply with the spectra of the ordinary 2-acetimido-2-deoxy sugars. Unlike the two amide residues of chitobiose, the chromophores of diacetamido sugars are sufficiently close to interact by mutual coupling much as occurs between adjacent amide residues of a regular polypeptide. The CD spectra of at least two biochemically interesting diacetamido sugars have appeared in the literature. 2,3-Diacetamidoglucose isolated from the lipopolysaccharide of several strains of photosynthetic bacteria has been studied by Keilich et al.[5] They report a spectrum having a strong negative band at 198 nm and a weak positive band near 222 nm. By analogy to polypeptide CD spectra, we may assign the 198-nm band

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Table I. Results for 1,2-Diacetamidoglucose for $\phi_1 = \phi_2 = 120°$

transition	energy, cm^{-1}	rotational strength, D-μ_B
$\pi-\pi^*$ (1)	52 933	−2.13
$\pi-\pi^*$ (2)	52 328	2.08
n-π^* (1)	47 169	−0.029
n-π^* (2)	47 168	0.077

as the long-wavelength limb of an exciton band, presumably paired with a band of opposite sign near 180 nm. The positive CD at 222 nm thus arises from an n-π^* band which may be partially obscured by the strong $\pi-\pi^*$ exciton band.

A second compound which has a CD spectrum of this same type is of substantial biochemical importance since it forms the juncture between peptide and the carbohydrate chain of the "serum-type" glycoproteins. Coduti et al.[4] have reported the CD of the glycopeptide linkage compound 2 acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (β-Asn-GlcNAc) as a function of pH. From the absence of a strong dependence of the CD spectrum on pH it was inferred that the amino and carboxyl function of the amino acid play only a minor role in determining the CD in the 190–220-nm region. Therefore the CD spectrum of β-Asn-GlcNAc, which shows a weak positive band near 210 nm along with a strong positive band at 195 nm and a strong negative band at 178 nm, may likewise be interpreted in terms of an exciton splitting in a vicinal diacylamido sugar.[4]

In order to interpret these Cotton effects in terms of the conformation of these compounds, we employ a theory introduced by Bayley, Nielsen, and Schellman[6] originally intended for use in dipeptides. This method, which we will call BNS, includes the influence of both the n-π^* transitions as well as the mutual coupling of the strong $\pi-\pi^+$ bands in a self-consistent manner. The large value of the molar ellipticity reported in the experiments cited above implies that these vicinal diacylamido sugars are rather rigid and that the amide chromophores are held in a fixed relative position.[4,5] Therefore these molecules serve as a better test of the BNS method for trans amides than do ordinary dipeptides which exhibit conformational mobility. Secondly, comparison of our calculations for various geometries of 1,2- and 2,3-diacetamidoglucose with experiment suggests some conclusions about the conformations of β-Asn-GlcNAc as well as 2,3-diacetamidoglucose.

II. Method

In the method of BNS a matrix containing the energies of interaction of the two electronic transitions (n-π^* and $\pi-\pi^*$) on the two amide residues is calculated. The elements of the resulting 4 × 4 matrix may be readily interpreted in terms of perturbation theory of CD. For example, the energy of interaction of the n-π^* and $\pi-\pi^*$ transitions of one amide with the static electric field of the other residue is the mixing parameter for a perturbation theory calculation of the one-electron Cotton effect.[1,7] A second type of matrix element involves the coupling of the n-π^* transition of one amide with the $\pi-\pi^*$ of the second. This effect, which is shown to be significant in dipeptides, is called the μ-m mechanism by BNS. Matrix elements substantially larger than the first two types are calculated for the interaction of the strong $\pi-\pi^*$ transitions on the two amides. This matrix element is responsible for the exciton splitting of the originally degenerate $\pi-\pi^*$ bands into two separate bands in the Kuhn–Kirkwood mechanism.

In these studies, we have followed the procedure of BNS for calculating the matrix elements in which monopole charges are assigned to approximate the appropriate charge distributions. Diagonalization of the energy matrix leads to four new states, two of which at nearly the same energy are mainly n-π^* in nature. The other two states, mainly $\pi-\pi^*$ in nature, are split by an energy approximately equal to twice the interaction energy between the two $\pi-\pi^*$ transitions. The rotational strengths for these latter two bands are nearly equal in magnitude but opposite in sign, yielding an approximately conservative CD spectrum in the region of the $\pi-\pi^*$ transition.

A computer program capable of generating Cartesian coordinates of the atoms of any molecule from bond distances, bond angles, and dihedral angles was used to generate distances for the monopole energy calculation. The state vectors resulting from the diagonalization were used to construct the rotational strengths of each state. We then used this method to reproduce the calculations of BNS for trans,trans dipeptides as a function of the dihedral angles ϕ_{CN} and ϕ_{CC} which describe the standard conformations of dipeptides.

In the Appendix to this paper we verify quantitatively the statement of BNS that when the bandwidth of the $\pi-\pi^*$ transition is large compared to the exciton splitting, the only significant observable parameter for exciton bands is the rotational couple. Thus in our results we report the rotational couple defined by BNS as the rotational strength of the lower energy of the two states which is mainly $\pi-\pi^*$ in nature multiplied by the splitting between the states in nanometers. Also following BNS, we report only the sum of the rotational strength for the two states that are mainly n-π^* in nature. As a result of the small difference in their energy, these two rotational strengths are not separately observable quantities. Thus this theory yields two independent parameters which may be compared with experiment.

III. Results

Geometry of the Sugar. The $^4C_1$ chair conformation, which places all nonhydrogen substituents of β-glucopyranose in an equatorial orientation, was selected for the calculations reported here. The second major geometric feature which determines the calculated result is the relative orientation of the amide planes. Therefore we have calculated the rotational strengths as a function of the dihedral angles which define rotation about the bond between the ring carbon atom and the amide nitrogen. In 1,2-diacetamidoglucose, we define ϕ_1 as the dihedral angle described by the four atoms C(amide), N(amide), C-1, and C-2 following the convention in which $\phi_1 = 0$ is the eclipsed conformation and right-handed screw rotation describes a positive dihedral angle. Similarly, ϕ_2 in 1,2-diacetamidoglucose is described by the four atoms C-1, C-2, N(amide), and C(amide).

Using these definitions $\phi_1 = \phi_2 = 120°$ represents a conformation in which the amide protons are trans to their respective ring C–H protons. The calculated transition energies and rotational strengths for this conformation are reported in Table I. Since these eight parameters are not independently observable, we have prepared maps of the two experimentally observable parameters as a function of the amide dihedral angles ϕ_1 and ϕ_2. Figure 1 illustrates the total n-π^* rotational strength, the sum of the rotational strengths of n-π^* (1) and n-π^* (2) of Table I. Figure 2 illustrates the rotational couple in nanometer–Debye–Bohr magnetons. The values in Figures 1 and 2 are reported to only one significant figure, which represents a generous estimate of the precision of optical activity calculations of this type.

At some values of ϕ_1 and ϕ_2 extremely close contacts between atoms of the two amides occur resulting in quite unrealistic values of calculated energies and rotational strength. We have therefore applied a test for steric contacts between atoms of the amides, rejecting conformations with contacts closer than the "extreme close contacts" given by Ramachandran and Sasisekharan.[8] The excluded conformations are marked by shaded regions in Figures 1 and 2.

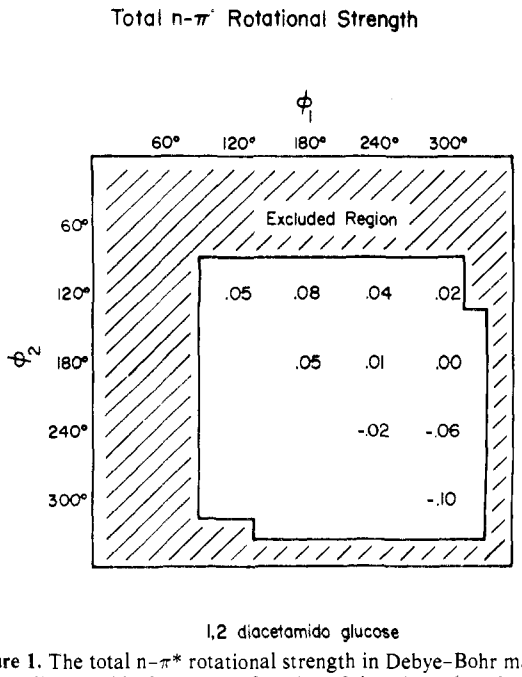

Figure 1. The total n-π^* rotational strength in Debye-Bohr magnetons for 1,2-diacetamidoglucose as a function of the dihedral angles defining the amide orientation.

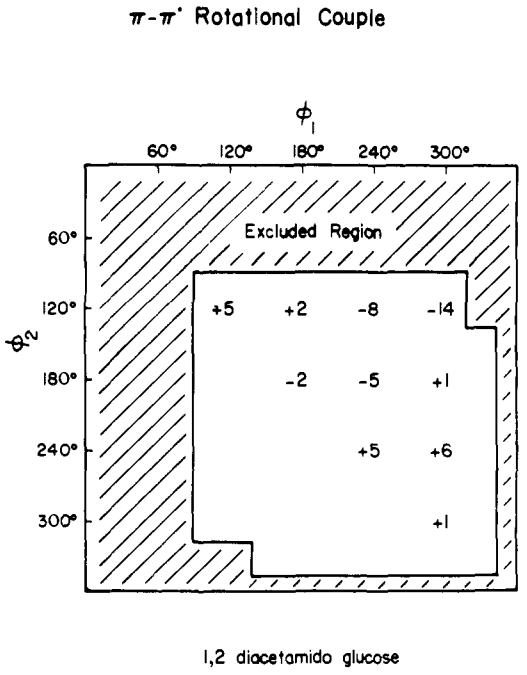

Figure 2. The π-π^* rotational couple in Debye-Bohr magneton-nanometers for 1,2-diacetamidoglucose as a function of the dihedral angles defining the orientation of the amide residues.

Since the amide residues of the diacetamido sugar are arranged head to head rather than in the head to tail configuration of a peptide, the symmetry of the rotational maps (Figures 1 and 2) differs from that reported by BNS for peptides. Specifically there is a C_2 rotational symmetry axis relating the C_1–N bond to the C_2–N bond. Therefore if $\phi_1 = \phi_2$ the atoms of one amide are related by this C_2 symmetry axis to the atoms of the other amide. Moreover, variations in ϕ_1 are equivalent to variations of ϕ_2 so the maps of Figure 1 and 2 are symmetric about the diagonal, $\phi_1 = \phi_2$.

As a consequence of the symmetry of the sugar ring, the results of Figures 1 and 2 may be applied to 2,3-diacetamidoglucose. Defining the dihedral angles of the amides by C(amide), N(amide), C-2, C-3 and by C-2, C-3, N(amide), C(amide) a conformation of 2,3-diacetamidoglucose becomes the mirror image of 1,2-diacetamidoglucose in which the signs of the amide dihedral angles are reversed. For example, 2,3-diacetamidoglucose with dihedral angles of $-120°$ has both amide protons trans to their respective ring CH protons and rotational strengths of the same magnitude but sign opposite to those of Table I. Therefore Figures 1 and 2 may be used to describe 2,3-diacetamidoglucose by changing the signs of both the dihedral angles and of the rotational strengths.

IV. Discussion

We demonstrate in the Appendix to this paper that the experimental value of the rotational couple for β-Asn-GlcNAc is +4.8 D-μ_B-nm. The overlapping of the long-wavelength band with the n-π^* CD band makes a precise estimate of the rotational strength of the latter difficult but it is presumably a positive value with a magnitude of approximately 0.05 D-μ_B. These values agree with the calculated results only for ϕ_1 and ϕ_2 near 120°. This conformation places the permanent dipole moments of the two amide residues in an antiparallel conformation in which their energy of interaction is negative. Moreover, this orientation is consistent with that found for β-Asn-GlcNAc in the solid state by means of x-ray crystallography.[9]

If 2,3-diacetamidoglucose were to have the same amide orientation as that of β-Asn-GlcNAc, then one would expect its CD spectrum to be the negative of the latter. The experimental results do not conform to this simple picture. From the data of Keilich et al.[5] we may estimate the rotational couple for 2,3-diacetamidoglucose to be −14.2 D-μ_B-nm but the rotational strength of the n-π^* transition is a small positive value, perhaps +0.02 to +0.05 D-μ_B.

It is possible that some one-electron effects not accounted for in this theory could make a large positive contribution to the n-π^* rotational strength causing the CD observed for 2,3-diacetamidoglucose to be positive for the conformation with amide protons trans to their respective ring protons. On the other hand, the calculations of Yeh and Bush[1] show that the only one-electron contributions as large as that calculated with the present model are contributions of the static field of the C-3 hydroxyl, a group which is absent in 2,3-diacetamidoglucose. Therefore we prefer to interpret the present calculations literally and seek a conformation for which the calculated results agree with experiment. Examination of Figures 1 and 2 with the signs reversed reveals that a large negative rotational couple in combination with a positive n-π^* rotational strength are predicted for the conformation with dihedral angles near −300° which places both amide protons cis to their respective ring protons. Since this conformation is not sterically crowded and the amide static dipole moments are antiparallel there is no obvious argument against it. Thus the CD suggests that the amides are oriented quite differently in these two apparently similar diacylamino sugars.

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Appendix[13]

It has been observed that when two broad CD bands of equal magnitude and opposite sign overlap, the resulting CD curve has maxima and minima which have magnitudes proportional to the splitting between the two bands.[10] Therefore, in coupled oscillator Cotton effects, the splitting and rotational strength will not be separately observable if the monomer absorption bandwidth is large compared to the splitting. The rotational

couple, the product of the splitting with the rotational strength, becomes the only experimental observable for the exciton bands of polypeptides and polynucleotides as well as the diacetamido sugars described in the accompanying paper.

In the case of Gaussian bands, the observed CD spectrum is given by

$$[\theta] = K\{\exp(-[(x - A)/\Delta]^2) - \exp(-[(x + A)/\Delta]^2\} \quad (1)$$

x is the difference $(\lambda - \lambda_0)$ between the observing wavelength and the monomer absorption wavelength, λ_0. A is the exciton splitting; K is proportional to the exciton rotational strength and Δ is the half bandwidth of the monomer absorption at $1/e$ of the maximum. The maxima and minima of this curve occur at values of $x = \lambda - \lambda_0$ satisfying

$$\ln\{(x/A + 1)/(x/A - 1)\} = 4Ax/\Delta^2 \quad (2)$$

Since solutions for this equation (2) exists only for $x/A > 1$, the experimentally observed extrema in the CD must always be at a separation greater than the calculated exciton splitting. Although solutions of eq 2 can generally be found graphically, for $x/A \gg 1$, a series expansion of eq 1 yields more insight. Setting $\delta = x/A$ and assuming that $\delta \gg 1$

$$[\theta] = K[4(A/\Delta)^2\delta]\,[1 - (A/\Delta)^2\delta^2 + \tfrac{1}{2}(A/\Delta)^6\delta^4 + \ldots] \quad (3)$$

The second term in brackets may be summed and written as an exponential.

$$[\theta] = K[4(A/\Delta)^2\delta]\exp[-(\delta A/\Delta)^2] \quad (4)$$

The extrema of eq 4 which is valid for $x/A \gg 1$ occur at

$$x_{max} = \pm\Delta/\sqrt{2} \quad (5)$$

Numerical comparison of the extrema calculated by eq 2 and by eq 5 indicates that the approximation eq 5 is valid for x/A as small as 2, a case for which the observed extrema are very nearly the splitting, A.

Substituting eq 5 into eq 4 we arrive at a formula showing that the observed maximum of the CD spectrum is proportional to the product of the splitting (A) with the rotational strength (K)

$$[\theta]_{max} = 2\sqrt{2}(KA/\Delta)\exp(-\tfrac{1}{2}) \quad (6)$$

For the case of $[\theta]_{max}$ in units of molar ellipticity, the rotational strength (R) in Debye-Bohr magnetons, and the splitting, the monomer absorption wavelength, and the bandwidth, all in nanometers, K has been evaluated by Moscowitz.[11]

$$K = 7514.42\,R\lambda_0/\Delta \quad (7)$$

Bayley et al.[6] define the rotational couple as the exciton rotational strength times the difference in wavelength of the two exciton bands. Thus the rotational couple in Debye-Bohr magneton-nanometers is given by the rotational strength times twice the wavelength displacement, A.

$$\text{Rotational couple} = 2AR = [\theta]_{max}\Delta^2/(6445\lambda_0) \quad (8)$$

For the amide absorption band at $\lambda_0 = 190$ nm, the absorption bandwidth, Δ, is approximately 14 nm. The CD data of Keilich et al. give $[\theta]_{max} = 89\,100$ for 2,3-diacetamidoglucose which leads to a value of -14.1 for the rotational couple. Coduti et al. report $[\theta]_{max} = +30\,000$ for β-Asn-GlcNAc corresponding to $+4.8$ for the rotational couple.

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(13) The Appendix was prepared with the assistance of Dr. Anthony Duben.