Conformation and Circular Dichroism of Oligosaccharides and Model Glycosides containing Neuraminic Acid (5-Acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulopyranosonic Acid) Residues

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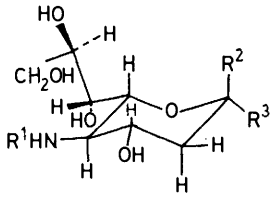
C.d. spectra have been recorded for *N*-acetylneuraminic acid, a widely occurring component of glycoproteins and glycolipids of animal cells and tissues, as well as for some of its synthetic and naturally occurring glycosides and related compounds. Principal spectral features are systemised in terms of the accessible chromophores, and the geometry and flexibility of glycosidic linkages. The main c.d. band, centred below 200 nm, is attributed to the acetamido group, and shows little structural or conformational sensitivity. Smaller spectral features at higher wavelength (around 225 nm) arise from the $n \rightarrow \pi^*$ transition of the carboxy chromophore, and provide a convenient index of glycosidic configuration and conformation. Thus α-linked glycosides of *N*-acetylneuraminic acid show a negative band, while β-glycosides give rise to a positive peak. In both cases the magnitude of the band is increased by restricted mobility. The planar symmetry rule developed by Listowsky is even more successful in predicting the sign of observed optical activity in sterically unambiguous fused ring lactone systems than the earlier and more complex lactone sector rule. Application of the planar rule provides a satisfactory unified interpretation of the observed c.d. behaviour of the carboxy chromophore of *N*-acetylneuraminic acid and its derivatives, in terms of established conformational principles. C.d. of the tetrasaccharide glycitol derived from the major cell surface glycoprotein of human erythrocytes, shows perturbations additional to those that occur between adjacent residues in the covalent sequence, suggesting that non-bonded interactions between branches of glycoproteins can be relevant to their tertiary structure.

OVER the last decade the implication of membrane glycoproteins in animal cell behaviour has become increasingly evident. These macromolecules have a polypeptide backbone to which oligosaccharide sidechains are linked through their reducing ends. The carbohydrate sequences normally contain up to about a dozen sugar units, do not show the regular periodic structures typical of many other carbohydrate components of biopolymers, and may be branched. Frequently a non-reducing terminal residue is 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulopyranosonic acid (1), which we will refer to subsequently by its trivial name of *N*-acetylneuraminic acid. Thus this sugar is on the outer surface of glycoprotein molecules which themselves are outermost structures of the cell, and it is therefore in the region in which first contact is made with outside stimulating agents. Presumably it is for this reason that its removal often alters cell response to



(1) α-anomer: $R^1 = Ac, R^2 = CO_2H, R^3 = OH$
β-anomer: $R^1 = Ac, R^2 = OH, R^3 = CO_2H$

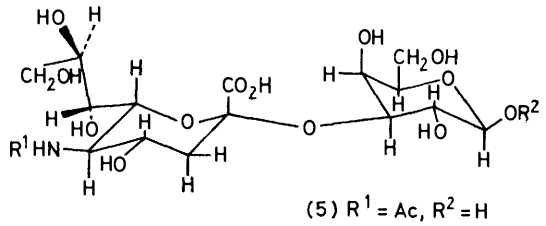
(2) $R^1 = Ac, R^2 = OMe, R^3 = CO_2H$
(3) $R^1 = H, R^2 = OMe, R^3 = CO_2H$
(4) $R^1 = Ac, R^2 = OH, R^3 = CH_2OH$

contact with other cells or inert surfaces. We here describe an investigation of the conformation and chiroptical properties of *N*-acetylneuraminic acid residues which we have undertaken as a companion to our study [1] of cell behaviour at the biological level.

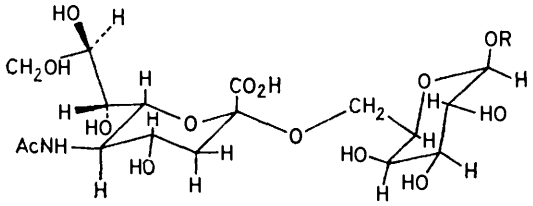
C.d. has proved a powerful technique for characterising carbohydrate composition, conformation, and interactions.[2] In particular c.d. studies [3] of model uronic acid glycosides have significantly advanced our understanding of the structure, ion binding behaviour, and association of polysaccharides containing these residues.[4] We have therefore extended this approach to oligosaccharides which model the occurrence of *N*-acetylneuraminic acid in membrane glycoproteins. The major glycoprotein of human erythrocytes has been the most extensively studied and characterised of membrance glycoproteins due principally to its ready availability and ease of extraction. The disaccharide units of *N*-acetyl-α-neuraminyl-(2 → 3)-D-galactose (5) and *N*-acetyl-α-neuraminyl-(2 → 6)-D-galactose (8) have been shown to be present as terminal sequences in both *O*- and *N*-linked oligosaccharide sidechains. In the present work we have studied the c.d. of a derivative of the main *O*-linked oligosaccharide of human erythrocytes which has been identified as a tetrasaccharide glycitol incorporating both these terminal sequences.

Although earlier studies were conducted,[5] only three recent papers have reported a systematic attempt to rationalise the c.d. behaviour of *N*-acetylneuraminic acid derivatives using modern equipment.[6-8] These disagree fundamentally in their findings on the c.d. behaviour of the carboxy chromophore. We have therefore prepared and studied several key model compounds to resolve this disagreement. We used *N*-acetyl-α-neuraminyl-(2 → 3)-β-D-galactopyranosyl-(1 → 4)-D-glucopyranose, [*N*-acetyl-α-neuraminyl-(2 → 3)-lactose (6)], and *N*-acetyl-α-neuraminyl-(2 → 6)-lactose (9) as model compounds, since they are conveniently isolated from human [9] and bovine [10] milk. Recent work [11] on human

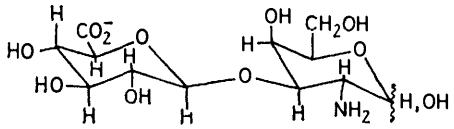
milk suggests that these derivatives are not metabolites of glycoproteins or glycolipids, but are biosynthesised from lactose.



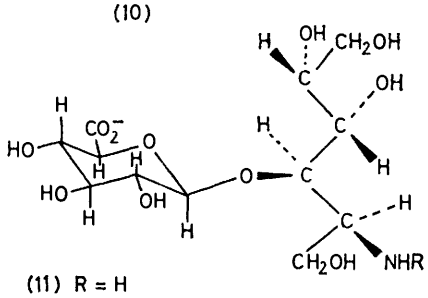
(5) R^1 = Ac, R^2 = H
(6) R^1 = Ac, R^2 = 4-D-Gp
(7) R^1 = H, R^2 = 4-D-glucitol



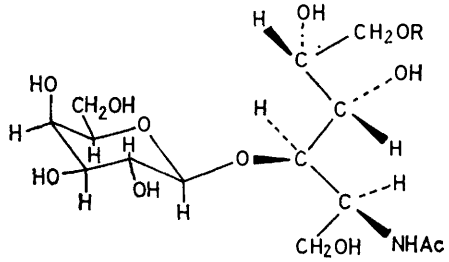
(8) R = H
(9) R = 4-D-Gp



(10)



(11) R = H
(12) R = Ac



(13) R = *N*-acetyl-α-neuraminyl-2-

Recently Listowsky [12,13] proposed a simple rule to rationalise the c.d. of α-substituted carboxylic acids, following Klyne's earlier sector rule [14] for the sign of the Cotton effects observed for a large number of sterically rigid fused ring lactones. We have reviewed all the spectral evidence adduced in support of Klyne's rule, and find that it is even more compatible with Listowsky's much simpler treatment, which we therefore suggest should be adopted in future. In our present study it provides a reasonable and consistent interpretation of the behaviour of the carboxy chromophore, and in particular affords a convenient method of distinguishing between α and β linked glycosides of *N*-acetylneuraminic acid.

EXPERIMENTAL

General.—C.d. spectra were recorded on a Cary 61 c.d. spectropolarimeter (1 mm path length cell; 10 s integration period). Sample temperature was regulated using a Haake thermocirculator and thermostatable cell holder. I.r. spectra were recorded on a Perkin-Elmer grating spectrophotometer 257, and the method of Bociek and Welti [15] was used to distinguish carboxylate salts from free acid, ester, or lactone. Samples were prepared by dissolving the carbohydrate (20 mg) in the free acid form in D_2O (1 ml) and the pH was adjusted if necessary by dropwise addition of DCl (0.1N) or NaOD (0.1N), or by buffering with NaH_2PO_4 and Na_2HPO_4.[15] Reference blanks were prepared in the same manner, but without the carbohydrate. Solid samples for i.r. were prepared as KBr microdiscs.

The method of Clamp [16] was modified to produce gas chromatograms of trimethylsilylated methyl glycosides. Chromatograms were run on a 150 cm column of OC-1 (3%) on 60—80 mesh Gas Chrom Q with a nitrogen flow of 60 ml min^{-1}, and a temperature programme of 136° for 10 min followed by a 2° min^{-1} rise to 210°. The internal standard was *meso*-inositol and results were corrected on the basis of recovery experiments for *N*-acetylneuraminic acid, galactose, and glucitol.

Mass spectra were recorded with the A.E.I. MS902 mass spectrometer, at an accelerating voltage of 8 kV, ion source temperature of 200° and 70 eV. Trimethylsilyl derivatives were prepared by using pyridine–trimethylchlorosilane–*NO*-bis(trimethylsilyl)acetamide (400 : 1 : 100). Methylation was by the method of Hakomori.[17] A Perkin-Elmer 241 polarimeter at ambient temperature was used to measure $[\alpha]_D$ values. Dry hydrazine was prepared as described by Fieser and Fieser.[18]

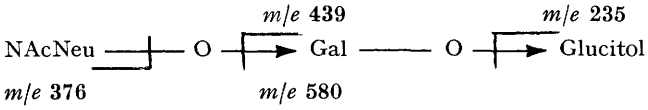
T.l.c. plates were prepared with Merck silica gels HF (type 60) and the solvent was butanone–methanol–N-acetic acid (12 : 3 : 5). Mobilities were measured relative to *N*-acetylneuraminic acid (R_N). Lactones were detected as hydroxamic acids by reaction with iron(III) nitrate,[19] and amines with ninhydrin (2%) in ethanol. *N*-Acetylneuraminic acid and its derivatives were detected using a spray containing resorcinol (0.2 g), concentrated hydrochloric acid (80 ml), and copper sulphate (0.1M, 250 ml), made up to a total volume of 100 ml, following the method of Svennerholm [20] and the plates developed by heating at 130° for 15—20 min. As a general detection agent, t.l.c. plates were also sprayed with 10% sulphuric acid then heated.

^1H N.m.r. spectra were recorded on three instruments: Varian HR-300 with superconducting magnet, Varian XL-100-15, both operating in the Fourier transform mode, and the Perkin-Elmer R12B n.m.r. spectrometer.

Compounds.—*N*-Acetylneuraminic acid (1) was from Koch–Light. The methyl β-glycoside of neuraminic acid

(3) was purchased from Boehringer Mannheim and its *N*-acetate (2) was prepared by subsequent *N*-acetylation using the method of Roseman and his co-workers.[21] T.l.c. confirmed that the starting material (R_N 0.7) has been converted to a single product (R_N 0.9). The mass spectrum after trimethylsilylation (sample temperature 150°) was consistent with the expected product showing the molecular ion (*m/e* 755) and peaks at *m/e* 740 ($M^{+\cdot} - CH_3$), 724 ($M^{+\cdot} - OCH_3$), 655 [$M^{+\cdot} - HOSi(CH_3)_3$], 638 [$M^{+\cdot} - CO_2Si(CH_3)_3$], 550 [$M^{+\cdot} - CH_2{\cdot}OSi(CH_3)_3{\cdot}CH{\cdot}OSi(CH_3)_3$], and 488 [$M^{+\cdot} - CH_2{\cdot}OSi(CH_3)_3{\cdot}CH{\cdot}OSi(CH_3)_3{\cdot}CH{\cdot}OSi(CH_3)_3$]. Chondrosine (10) from shark cartilage chondroitin 4-sulphate was a gift from Dr. A. H. Olavesen. Borohydride reduction [22] to (11) was followed by *N*-acetylation to (12), by treatment with acetic anhydride.[23] *N*-Acetyl-α-neuraminyl-(2 ⟶ 3)-lactose (6) and *N*-acetyl-α-neuraminyl-(2 ⟶ 6)-lactose (9) were isolated from bovine colostrum, following the method of Schneir and Rafelson [10] except that the final purification was by aqueous elution from a Sephadex G10 column, rather than by ether–methanol precipitation. α-Neuraminyl-(2 ⟶ 3)-lactitol (7), $[\alpha]_D$ 0° (*c* 0.39, water), was prepared from *N*-acetyl-α-neuraminyl-(2 ⟶ 3)-lactose (20 mg) by first reducing with sodium borohydride using the standard procedure,[22] except that treatment with cation exchange resin was at 4 °C, then de-*N*-acetylation [24] with hydrazine (0.5 ml) and hydrazine sulphate (5 mg). Final purification was by elution with water from a Sephadex G-10 column monitored by t.l.c., followed by freeze drying. ^{1}H N.m.r. confirmed the absence of acetate. After methanolysis, *N*-acetylation, and trimethylsilylation, g.l.c.[16] showed the derivatives of galactose, glucitol, and neuraminic acid in the ratio 1.0 : 1.0 : 0.8. *N*-Acetylation of the trisaccharide glycitol itself gave *N*-acetyl-α-neuraminyl-(2 ⟶ 3)-lactitol (7), $[\alpha]_D$ +24° (*c* 1.18, water). Analysis as above showed derivatives of galactose, glucitol, and *N*-acetylneuraminic acid in the ratio 1.0 : 1.0 : 0.9. The mass spectrum of the permethylated trisaccharide (Table; sample temperature

Mass spectrum of permethylated *N*-acetyl-α-neuraminyl-(2 ⟶ 3)-lactitol



m/e	Assignment
831	$M^{+\cdot}$
816	$M^{+\cdot} - CH_3$
786	$M^{+\cdot} - CH_2OMe$
772	$M^{+\cdot} - COOMe$
756	$M^{+\cdot} - CH_2OMe - 2Me$
580	See above
439	See above
376	See above
235	See above

150°) was identical to that of an authentic sample [25] of permethylated *N*-acetyl-α-neuraminyl-(2 ⟶ 3)-lacitol. The positions of the peaks at *m/e* 376, 756, and 772 were located accurately using the 369 and 743 peaks of perfluorokerosene as markers. The *O*-linked tetrasaccharide (13) of the major glycoprotein of human erythrocytes was prepared by alkaline borohydride treatment of the sialoglycopeptide fragment liberated on digestion with trypsin, after the method of Winzler and his co-workers.[26] It was isolated by chromatography on Sephadex G-25, converted to the free acid form on Dowex 50-8X, and characterised by sugar analysis, as described above. The identity and purity of all compounds, major intermediates, and derivatives were further confirmed by high resolution ^{1}H n.m.r.

RESULTS AND DISCUSSION

Glycosides of N-*Acetylneuraminic Acids.*—Figure 1 shows the c.d. spectra we obtained for two typical glycosides, α and β linked respectively. Closely similar spectra have been reported by Keilich *et al.*,[6] who attributed the large positive band at low wavelength to

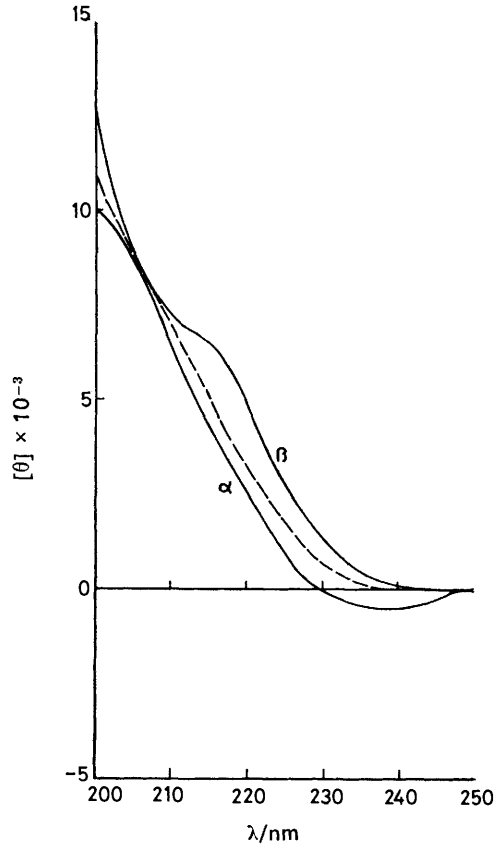


FIGURE 1 Circular dichroism spectra of glycosides of *N*-acetylneuraminic acid (9) and (2), in which the glycosidic linkages are α and β respectively. The broken line (from ref. 6) shows the c.d. of (4), which is the carboxy-reduced derivative of (2) indicating that the trough and shoulder in the other spectra have their origin in the carboxy chromophore

the acetamido group, while assigning the smaller spectral features around 220 nm to the carboxy chromophore. To verify this they carboxy-reduced the methyl β-glycoside (2) and found that, as expected, the shoulder at higher wavelength was lost. We have further substantiated the assignment from the effect of pH, since ionisation fundamentally alters the nature of the carboxy chromophore by introducing an additional plane of symmetry as the two oxygen atoms become equivalent. This may produce radical changes in the c.d. behaviour, as in uronic acids,[3] whereas amide groups are unaffected by moderate pH changes.

Figure 2(a) shows spectral changes with pH which we have observed for *N*-acetyl-α-neuraminyl-(2 ⟶ 3)-

lactose (6), and for *N*-acetylneuraminic acid itself which we have shown by ^{1}H n.m.r. to exist predominantly in the β form (although not as previously suggested [6] exclusively β). It is evident that these changes are centred around the higher wavelength region, as expected.

In α-glycosides of *N*-acetylneuraminic acid the carboxy group is axially oriented, while for β it is equatorial. We might therefore expect its c.d. behaviour to be diagnostic of the linkage geometry which in turn is critical to the overall shape. From Figure 1 we might conclude, as suggested by Kielich *et al.* that the carboxy band in β-linked compounds is positive, while for α linkages it is negative. The results of Jennings *et al.*[8] partially support this interpretation, in that they also observed a long wavelength positive c.d. band in the methyl acetamidodeoxy-β-glycoside (2), although they saw no indication of a band of opposite sign in the c.d. of the α-anomer. Dickinson and Bush,[7] however, reported a strong *negative* band at 220 nm for the methyl β-glycoside (3) in direct disagreement with this generalisation. They do, however, comment that the result is ' somewhat surprising '. To resolve this discrepancy we have re-examined the c.d. of the methyl β-glycoside (3) and also prepared α-neuraminyl-(2 → 3)-lactitol (7). The spectra are shown in Figure 2(b), and are in complete agreement with the rule proposed by Keilich *et al.* Furthermore the changes with pH agree in sign and magnitude with those shown in Figure 2(a), showing

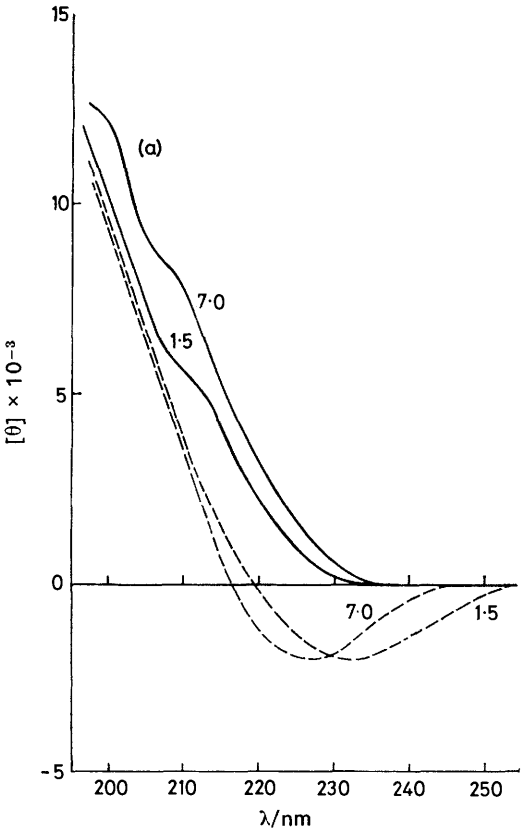


FIGURE 2

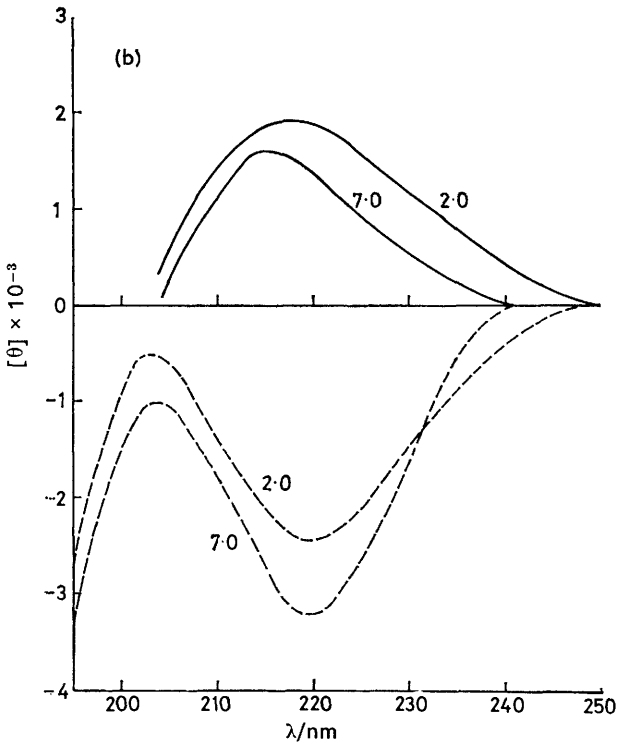


FIGURE 2 Effect of ionisation on the c.d. of the carboxy chromophore in neuraminic acid and derivatives, with pH values shown on the curves. (a) In the presence of the acetamido chromophore: (– – –) the α-glycoside (6) and (——) *N*-acetylneuraminic acid (1) in predominantly the β form. (b) In the absence of other accessible chromophores: (– – –) the α-glycoside (7) and (——) the β-glycoside (3). Comparison of (a) and (b) confirms that the large positive c.d. band centred below 200 nm in acetamido derivatives does arise from the amide chromophore

consistency in chiroptical behaviour of the carboxy chromophore before and after *N*-deacetylation. We cannot account for the discrepancy between our spectrum and that reported by Dickinson and Bush.

We could, of course, place much more confidence in this empirical correlation between c.d. and glycosidic linkage if it were shown to be in accord with other evidence relating carboxy c.d. to molecular structure. Schellman [27] has demonstrated the effectiveness of examining the disposition of other atoms or groups in a molecule about the symmetry planes of the chromophore. The rationale of this approach is that whatever effect a particular substituent has in inducing optical activity in one position, it will have an equal and opposite effect when present in the mirror image position relative to the chromphore. Although less rigorous than an approach to c.d. interpretation through molecular orbital calculations, such spatial rules have proved very effective in correlating the sign of observed Cotton effects with the geometry of the molecule.[28] The best known and most firmly established is the octant rule [29] for ketone $n \rightarrow \pi^*$ transitions, but several effective unifying rules have been developed for other chromophores.[30] In particular two rather different treatments have been proposed for the carboxy $n \rightarrow \pi^*$ transition. The first of

these was the lactone sector rule developed by Klyne *et al.*[14] This is summarised in Figure 3(a), and involves looking at the molecule along the C–C bond joining the carboxy carbon atom to the rest of the molecule. It was suggested that only groups lying in specific narrow sectors symmetrically disposed around the chromophore are effective in inducing optical activity, the sign of the resultant Cotton curve being predicted from the sign associated with the particular sector in which the group lies. More recently Listowsky[13] has proposed a rule, summarised in Figure 3(b), which simply states that groups on opposite sides of the plane of the carboxy group have opposite effects. In view of the simplicity of this approach we have re-examined the empirical

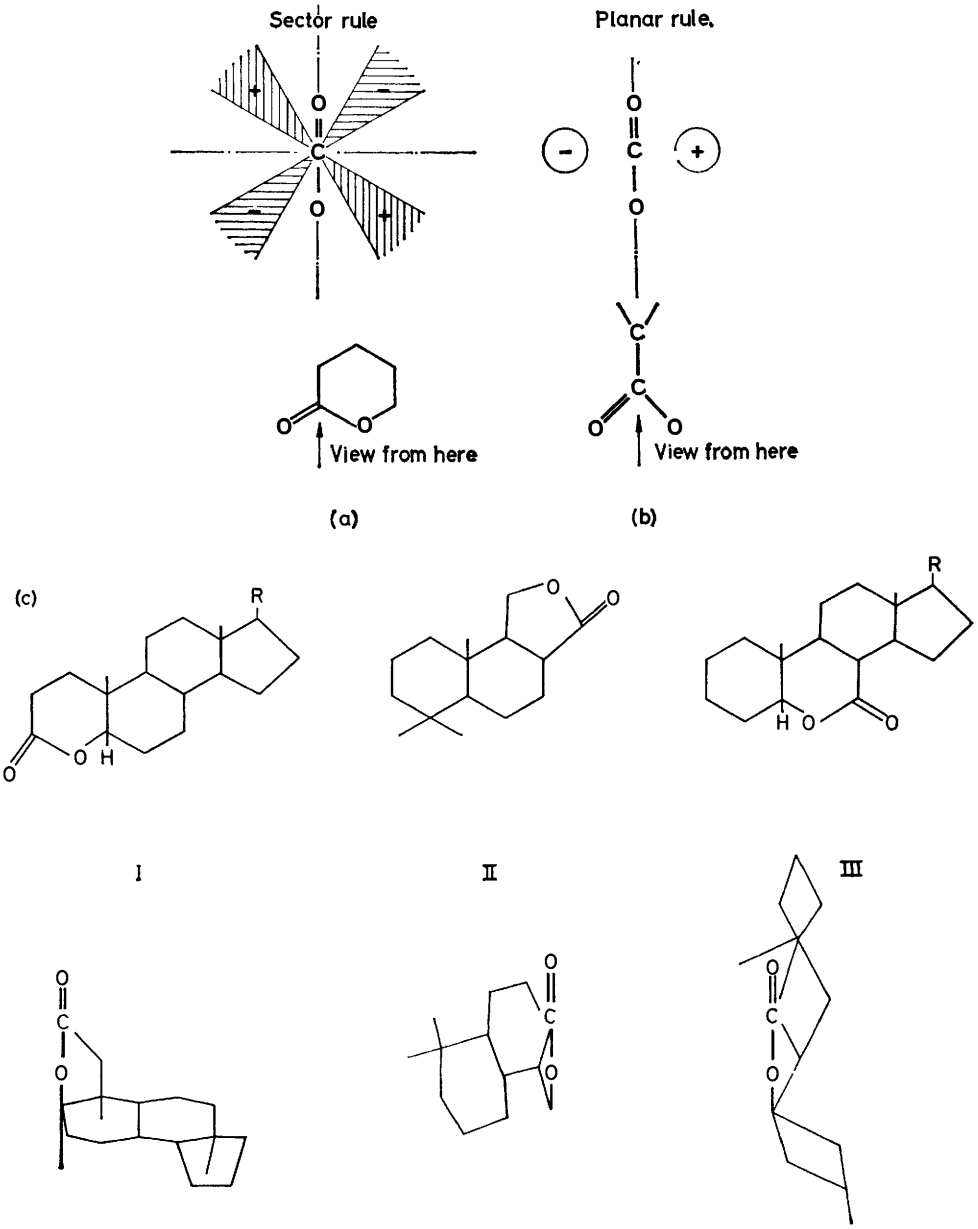


FIGURE 3 Symmetry rules for prediction of the sign of carboxy Cotton effects: (a) the lactone sector rule;[14] (b) the carboxy planar rule.[13] In both treatments the molecule is viewed along the plane of the carboxy group, as shown, and the sign of the predicted optical activity is determined by the spatial disposition of other groups around the chromophore, as indicated. (c) Application of the planar rule to the fused ring lactone systems used [14] to verify the sector rule shows that the simpler planar symmetry treatment is even more successful in rationlising signs of the observed optical activity. Thus for terminal ring lactones such as 3-oxo-4-oxa-5β-steroids (I) and 11-hydroxyiresan-8αβ-oic acid lactone (II) [structures (XVII) and (XXIV) in ref. 14], both approaches successfully predict the sign of the observed optical activity (positive and negative respectively), but when the lactone group is in a middle ring as in 7-oxo-6-oxa-5β-steroids (III) [structure (XVIII) in ref. 14], the sector rule is ambiguous while the planar rule correctly predicts the observed positive Cotton effect. In each case the upper figure shows the conventional structural formula, while the lower shows the appropriate projection for direct application of the symmetry rules outlined in (a) and (b)

data on which the sector rule was based, to see whether it is equally consistent with the planar rule. Our conclusions are summarised in Figure 3(c) from which it is evident that the planar rule is even more successful in rationalising the signs of the observed Cotton effects in terms of molecular structure. We now regard this rule as providing a broad based, reliable unification of the chiroptical behaviour of the carboxy $n\rightarrow\pi^*$ transition and have proceeded to use it to test our empirical correlation of glycosidic linkage configuration for *N*-acetylneuraminic acid with the sign of carboxy c.d.

It is evident that rotation of the carboxy group about the C(1)–C(2) bond will drastically alter the disposition of the rest of the molecule about the symmetry plane of the chromophore. Crystallographic studies of a wide range of carboxylic acids conclusively show that the preferred conformations have the carbonyl function of the carboxy group close to an eclipsed position.[31] In particular for α-hydroxy acids, eclipsing is invariably with the hydroxy oxygen. Listowsky has succeeded [13] in interpreting the c.d. behaviour of α-substituted carboxylic acids in terms of such preferred conformations.

In the particular case of *N*-acetylneuraminic acid it is not immediately evident whether eclipsing of the ring oxygen or the glycosidic oxygen would be expected, although crystallographic evidence,[32] shows that it is the glycosidic oxygen which is actually eclipsed in the solid state. In fact, as indicated in Figure 4, whichever oxygen is eclipsed, application of the planar rule predicts a positive c.d. band for β-glycosides and a negative band for α-glycosides. Thus even if the energy difference between the two rotational isomers is sufficiently small for the two to exist in equilibrium in solution, the effective conclusion is unchanged: the planar rule successfully predicts the observed linkage dependence of the c.d. of glycosides of *N*-acetylneuraminic acid.

As well as the gross dependence of c.d. sign on structure, several subtler correlations emerge. α-Hydroxycarboxylic acids show two c.d. bands attributable to the $n\rightarrow\pi^*$ transition, one at the normal position of 212 nm, and a smaller band centred at higher wavelength, around 225 nm. Listowsky [13] has attributed the normal band to the preferred rotational isomer where the α-oxygen atom lies in the plane of the chromophore, and the smaller, red shifted band to the less stable form in which C(3) is eclipsed as shown in Figure 5. Thus the presence of an oxygen atom in the conformation shown in structure (II) (Figure 5) appears to lower the transition energy,

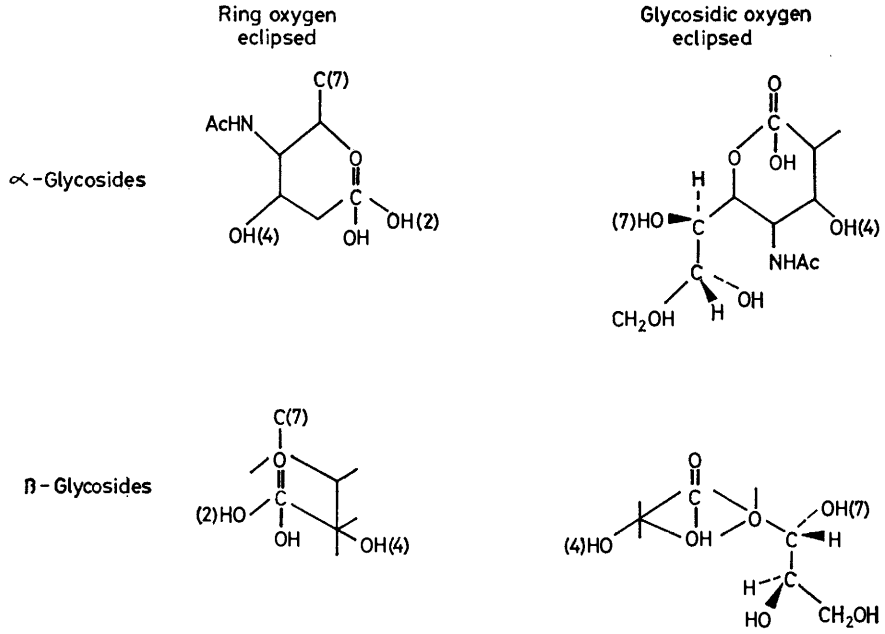


FIGURE 4 Application of the planar rule to the carboxy chromophore predicts negative c.d. behaviour for α-glycosides of neuraminic acid, and positive c.d. behaviour for β-glycosides, irrespective of which of the two likely orientations of the carboxy group is adopted. This is in agreement with the experimental evidence in Figures 1 and 2. The molecular projections used are as indicated in Figure 3

perhaps by stabilising the π^* excited state. It is evident that for either of the oxygen-eclipsed conformations of *N*-acetylneuraminic acid the oxygen atom which

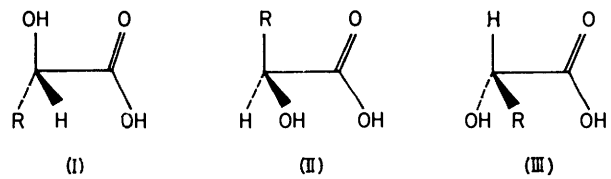


FIGURE 5 Eclipsed conformations of α-hydroxycarboxylic acids

is not eclipsed lies in this same orientation with respect to the carboxy group. This could then account for the fact that the $n\rightarrow\pi^*$ transition occurs at a significantly higher wavelength than is usual for other carboxylic acids.

The c.d. of *N*-acetyl-α-neuraminyl-(2 ⟶ 6)-lactose (9) is very similar to those recorded by Keilich *et al.*[6] for synthetic alkyl α-glycosides of *N*-acetylneuraminic acid, but as shown in Figure 2(b), the carboxy band of *N*-acetyl-α-neuraminyl-(2 ⟶ 3)-lactose (6) is appreciably enhanced. We suggest that while 6-linked lactose will have essentially the same aglycone behaviour as, say, an ethyl group, a 3-linked sugar presents much more steric interaction which could for example inhibit eclipsing of the glycosidic oxygen and hence favour the form in which the ring oxygen is eclipsed; in this rotamer (Figure 4), the remainder of the molecule lies more completely to one side of the plane of the chromophore.

The spectrum reported by Bush *et al.* for the methyl ester of neuraminic acid methyl β-glycoside (3) is extremely similar to that which we have obtained for the free acid glycoside, as shown in Figure 2(b). Similarly Keilich *et al.*[6] found little difference between the c.d. spectra of the ester and free acid forms of the corresponding *N*-acetates. We therefore conclude that esterification has little influence on the c.d. behaviour of *N*-acetylneuraminic acid derivatives. This is in accord with our observations [4] on polysaccharide systems such as pectin, where the degree of methyl esterification has little effect on c.d. at acid pH, although, of course, the salt and ester spectra may be very different.

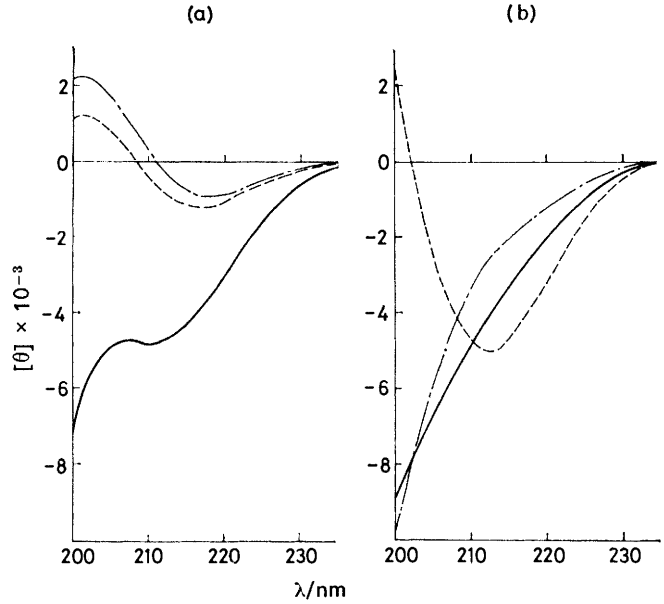


FIGURE 6 C.d. of 2-acetamido-2-deoxy-D-galactose and its derivatives: (a) (– – – –) chondrosine (10), (— · — · —) its glycitol (11), and (——) glycitol *N*-acetate (12); (b) (– – – –) 2-acetamido-2-deoxy-D-galactose, (— - —) 2-acetamido-2-deoxy-D-galactitol, (——) the estimated amide contribution to the c.d. of (12), showing the significant enhancement of ellipticity on introduction of substituents close to the chromophore. This spectrum was derived by subtraction of the spectrum for (11), where the carboxy group is the only accessible chromophore, from the spectrum for (12), where both chromophores are present

Oligosaccharide Structure.—We have applied the understanding developed in these studies of model glycosides of *N*-acetylneuraminic acid to interpret the c.d. of carbohydrate oligomers derived from the major membrane glycoprotein of human erythrocytes, in search of chiroptical effects which arise from secondary

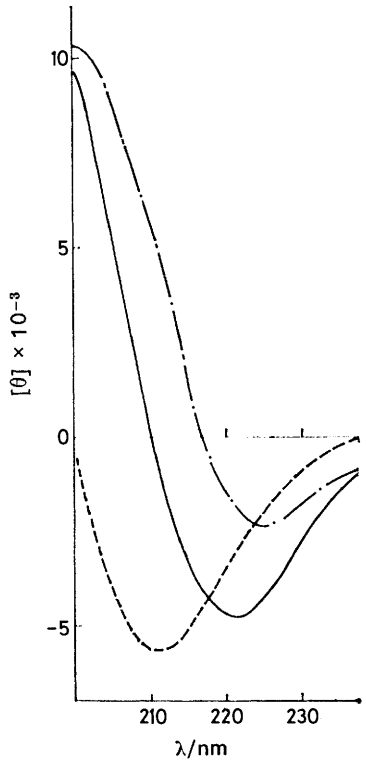


FIGURE 7 C.d. behaviour of the tetrasaccharide glycitol (13) derived from the *O*-glycosidically linked chains of the major membrance glycoprotein of human erythrocytes: (——) observed spectrum; (— - —) spectrum derived by linear combination of the c.d. behaviour of the individual chromophores in closely analagous local molecular environments, *i.e.* α-(2⟶3) and α-(2⟶6) linked *N*-acetylneuraminic acid [compounds (6) and (9) respectively, see Figures 1 and 2], and the amide chromophore in (12) as derived in Figure 6; (– – – –) the difference between these observed and calculated spectra, showing the enhancement in ellipticity due to additional interactions within the tetrasaccharide

structure. In addition to the chromophores of *N*-acetylneuraminic acid, this tetrasaccharide glycitol (13) contains a disubstituted 2-acetamido-2-deoxy residue which also contributes to c.d. Since previous chiroptical studies [33] have shown that the acetamido chromophore is very sensitive to local molecular geometry, we examined analogous model compounds in an attempt to estimate its contribution.

The disaccharide chondrosine [β-D-glucopyranosyluronic acid-(1 ⟶ 3)-2-amino-2-deoxy-D-galactose (10)] was reduced to the corresponding glycitol (11) then converted to the *N*-acetate (12). The c.d. spectra of these compounds [Figure 6(a)] show that the carboxy c.d. is slightly modified by conversion to the glycitol, and massive increase in negative ellipticity then occurs with *N*-acetylation. Since we know from extensive studies of the c.d. of uronic acid glycosides [4] that the spectral contribution of the carboxylate chromophore will be little affected by structural modification of the aglycone, subtraction of the spectrum of the glycitol (11) from

that of its *N*-acetate (12) should allow the c.d. contribution of the acetamido chromophore to be estimated. Direct comparison with corresponding monosaccharide spectra [Figure 6(b)] illustrates that there is some enhancement of ellipticity on introduction of bulky substituents in positions adjacent to the chromophore, as previously suggested.[33] By contrast, substitution on O(6) is unlikely to have any substantial effect on chiroptical behaviour of the acetamido chromophore.[33]

We can now attempt to predict an overall circular dichroism spectrum of the tetrasaccharide glycitol (13) by linear combination of the spectra of the constituent chromophores namely α-2,3 linked *N*-acetylneuraminic acid, α-2,6 linked *N*-acetylneuraminic acid, and 3-substituted 2-acetamido-2-deoxy-D-galactitol. As shown in Figure 7, however, it turns out that such a prediction is far removed from the observed c.d. spectrum, indicating steric constraints beyond those between adjacent residues in the covalent sequence. Inspection of a space-filling molecular model of the tetrasaccharide glycitol shows no obvious additional constraints on the freedom of movement of chromophores of the *N*-acetylneuraminyl residues, whereas the acetamido chromophore of the galactosaminitol residue probably becomes more crowded by the 3-*O*-linked side chain if, as seems likely, this adjusts its average position because of interactions with the 6-*O*-linked chain. The similarity in position, sign, and spectral form of the additional ellipticity to the spectrum of 2-deoxy-2-acetamido-D-galactose in Figure 6(b) would be consistent with this interpretation. Our results therefore argue that the acetamido chromophore is sensitive to additional conformational restrictions arising from the branched structure of the molecule. It seems likely that similar non-bonded interactions between branches may have an important influence on the secondary and tertiary structure of intact glycoproteins.

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