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CIRCULAR DICHROISM STUDIES ON α- AND β-KETOSIDES OF 5-ACETAMIDO-3,5-DIDEOXY-D-*glycero*-D-*galacto*-NONULOPYRANOSONIC ACID (*N*-ACETYLNEURAMINIC ACID) AND OF SOME OF ITS DERIVATIVES*

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ABSTRACT

The circular dichroism spectra of a number of *N*-acetylneuraminic acid derivatives in aqueous solution were studied. For all compounds, the Cotton effects were found to be in the spectral range of the acetamido and carboxyl chromophores. The c.d. curves of the methyl, ethyl, and allyl α-D-ketosides are characterized by a broad, positive band centered at $\lambda \sim 195$ nm with a slight skew towards the higher wavelengths and weak bands between λ 225 and 255 nm, whereas the methyl β-D-ketoside and the corresponding methyl ester show only an intense positive band with a broad shoulder in the same spectral range. 5-Acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-nonulopyranose, its methyl β-D-ketoside, and 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulopyranosonamide containing only the acetamido chromophore showed one single positive Cotton effect centered at $\lambda \sim 192$ nm. The c.d. spectrum of 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulopyranosonic acid confirms the β-D configuration of the free acid in aqueous solution, whereas the shape of the c.d. curve of *O*-(*N*-acetyl-α-D-neuraminopyranosyl)-(2→3)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose resembles that of the methyl, ethyl, and allyl α-D-ketosides **2–4**.

INTRODUCTION

The study of polysaccharides, such as heparin, chondroitin, and dermatan sulfates[1], and of immunochemically reactive oligo- and polysaccharides containing significant proportions of 2-acetamido-2-deoxy sugars[2] shows the usefulness of circular dichroism measurements for investigation of the structure of complex

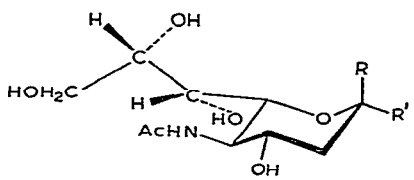
*The definition of the configuration at C-2 is based on the systematic name used in the title, as specified in the British–American Rules of Carbohydrate nomenclature (see W. Pigman and D. Horton, *The Carbohydrates*, 2nd ed., Vol. IA, Academic Press, New York, 1972, p. 55).

carbohydrate molecules. In this connection, the examination of the c.d. spectra of monosaccharide constituents of known configuration and conformation have been essential to interpret the results obtained with such complex oligo- and polysaccharides. Therefore, characteristics of the c.d. spectra in the u.v. region have been correlated with various structural aspects of uronic acids, 2-acetamido-2-deoxy sugars, and their glycosides, and tentative rules concerning the nature and linkage of the sugar residues in oligo- and polysaccharides have been proposed[2-4].

N-Acetyl-(and glycolyl)neuraminic acid and its *O*-acetyl derivatives are widely distributed in biological material, *e.g.*, in milk oligosaccharides, brain gangliosides, and the carbohydrate moieties of glycoproteins. The structure of these compounds, particularly the structural parameters that depend on the neuraminic acid constituents, are of interest for the specific biological function. In studies of the structure of gangliosides, attempts have been made to relate the optical parameters with the complex chemical structure, *e.g.*, the position of the *N*-acetylneuraminic acid residues[5,6]. The interpretation of the c.d. data of such complex, biological compounds, however, are complicated by the presence of 2-acetamido-2-deoxy-hexoses and of *N*-acetylneuraminic acid, both of which contain the acetamido chromophore. In addition, no detailed knowledge of the optical properties of simple *N*-acetyl-D-neuraminic acid derivatives has been reported so far. Therefore, the relationship between the specific structural parameters of neuraminic acid derivatives (*e.g.*, the type of configuration of the ketosidic linkage and the orientation of various substituents) and the c.d. spectra of these compounds have been investigated.

RESULTS

Methyl (**2**), ethyl (**3**), and allyl (**4**) α-ketosides of 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulopyranosonic acid (*N*-acetylneuraminic acid) (**1**) in aqueous solution exhibit intense positive Cotton effects at λ ~197 nm and weak bands between λ 225 and 255 nm, **2** with positive sign at λ 249 nm, **3** with negative and



1 R = OH , R' = CO_2H	6 R = OMe , R' = CO_2Me
2 R = CO_2H , R' = OMe	7 R = OH , R' = CH_2OH
3 R = CO_2H , R' = OEt	8 R = OMe , R' = CH_2OH
4 R = CO_2H , R' = $OCH_2CH{=}CH_2$	9 R = OH , R' = $CONH_2$
5 R = OMe , R' = CO_2H	10 R = CO_2H , R' = lactose

positive sign at λ 236.5 and 252.5 nm, respectively, and **4** with negative sign at λ 235 nm (see Fig. 1 and Table I). The profile of the c.d. bands centered at λ ~197 nm

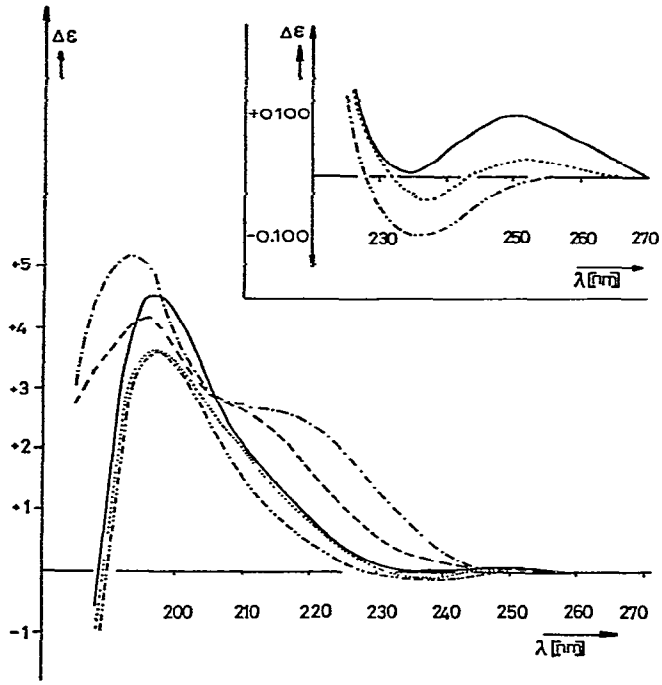


Fig. 1. C.d. spectra of **2** (———), **3** (·····), **4** (—·—), **5** (— — —), and **6** (—·—·—), in aqueous solution, pH 3.0–3.5, at 20° and concentration of 0.2–1%.

TABLE I

CIRCULAR DICHROIC ABSORPTION OF *N*-ACETYLNEURAMINIC ACID (**1**) AND ITS DERIVATIVES (**2–10**)

Compounds	*λ(nm)*	*Amplitude* (Δε)
1	193	+3.54
	200–210[a]	+2.52; +2.2
2	196.5	+4.55
	249	+0.10
3	197.5	+3.69
	236.5	−0.035
	252.5	+0.025
4	197.5	+3.63
	235	−0.10
5	195	+4.13
	210–215[a]	+2.55; +2.2
6	192.5	+5.19
	205–220[a]	+2.9; +2.4
7	192	+5.75
8	192	+5.01
9	194	+6.36
10	197.5	+3.25
	225	−0.450

[a]Shoulder.

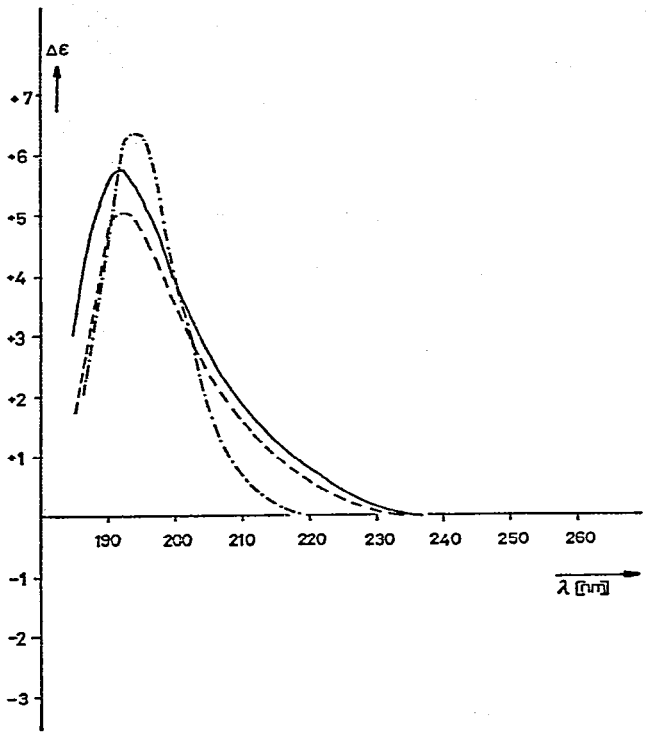


Fig. 2. C.d. spectra of 7 (———), 8 (— — —), and 9 (-·-·-·-) in aqueous solution, pH 3.0–3.5, at 20° and concentrations of 0.2–1%.

is asymmetric, indicating the overlap of two Cotton effects having opposite sign. The methyl β-ketosides (**5**) of **1** and of the corresponding methyl ester (**6**) show positive dichroic absorption; the maximum is observed at λ_{max} 193 nm and a broad shoulder, probably due to a further optical chromophore, is located between λ 205 and 220 nm (Fig. 1 and Table I). The c.d. spectra of 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-nonulopyranose (**7**), of its methyl β-D-ketoside (**8**) and of 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-nonulopyranosonamide (**9**), however, feature only positive ellipticity bands near λ 192 nm; in the region of longer wavelengths, neither a c.d. band nor a shoulder is found (Fig. 2 and Table I). Fig. 3 shows the c.d. spectra of *N*-acetylneuraminic acid (**1**) and *O*-(*N*-acetyl-α-D-neuraminopyranosyl)-(2→3)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose[*O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose] (**10**); **1** exhibits a broad positive band at λ 193 nm and a shoulder at λ 205–210 nm, whereas **10** shows a strong positive and a weak negative c.d. band centered at λ 197.5 and 225 nm, respectively. These c.d. data obtained with aqueous solutions having pH 3.0–3.5 are essentially independent of the concentration of the substances. The change of pH only influences the amplitudes of the c.d. bands.

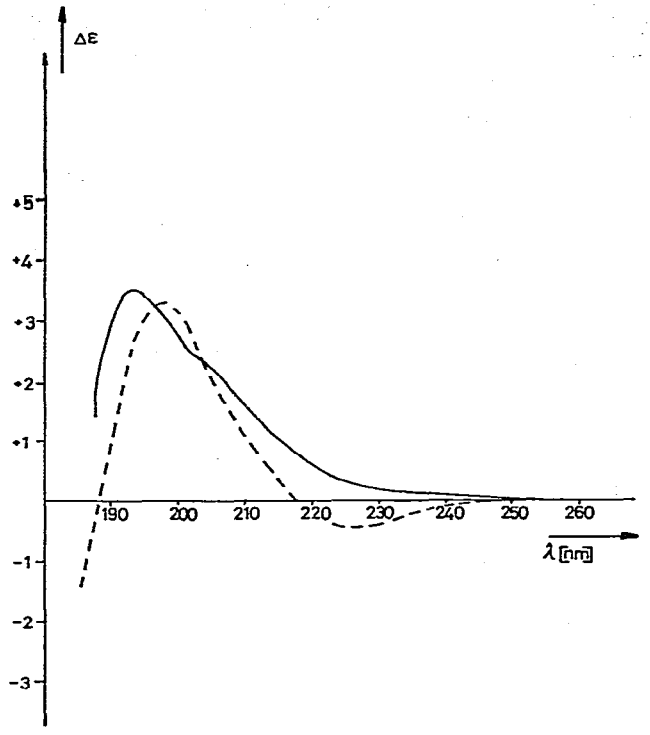


Fig. 3. C.d. spectra of **1** (———) and **10** (— — —) in aqueous solution, pH 3.0–3.5, at 20° and concentrations of 0.2–1%.

DISCUSSION

N-Acetylneuraminic acid (**1**) and its derivatives containing an *N*-acetyl and a carboxyl group exhibit Cotton effects in the spectral range of these chromophore transitions (between λ 190 and 255 nm). Both the α-D-ketosides **2**, **3**, and **4** and the β-D-ketosides **5** and **6** bring about positive c.d. bands around λ 195 nm, differing only in the values of ellipticity magnitude (see Table I). At a higher wavelength, however, the c.d. curves of the anomeric compounds are different, **2**–**4** exhibiting small positive or negative bands between λ 205 and 255 nm, **5** and **6** only a broad shoulder in the same spectral range (see Fig. 1 and Table I)*.

An earlier report[5] described positive c.d. curves for the water solution of both methyl α-D- and β-D-ketosides of **1**; for the methanol solution of these substances, negative and positive Cotton effects, respectively, were observed. These c.d. measurements were, however, not determined at wavelengths shorter than λ 220 nm, and their discussion was restricted to the range between λ 220 and 300 nm. The c.d.

*The weak bands at ~230–255 nm, as shown in the inset of Fig. 1, appear rather as overlaps of tails of bands centered further down. Thus, the extrema observed do not necessarily indicate transitions centered at the wavelengths measured.

spectra of the α-D- and β-D-ketosides measured in this laboratory on aqueous solutions show only small Cotton effects in the same wavelength region, but unequivocally characteristic differences (see Fig. 1 and Table I). At shorter wavelengths, however, the spectra differ only in the ellipticity magnitude of the bands. The reason for the different results observed in the two laboratories cannot be explained without a direct comparison of the samples. The spectra of **2**–**6** in methanol and in trifluorethanol solutions showed similar but amplified differences. A detailed discussion of the influence of solvents on the optical properties of derivatives of **1** will be reported later.

The c.d. curve of **1** shows the same pattern as those of the β-D-ketosides **5** and **6**, but with smaller dichroic absorption values (see Figs. 1 and 3, and Table I). Thus, the optical properties confirm the β configuration of the free acid in aqueous solution established by n.m.r. spectroscopy[7]. On the other hand, the shape of the c.d. curve of the trisaccharide **10** resembles that of the α-D-ketosides, except in one point: the negative c.d. band is shifted to shorter wavelengths (λ_{max} 225 nm) and is more pronounced than the corresponding band of the α-D-ketosides **3** and **4** (see Figs. 1 and 3, and Table I). The c.d. spectrum of **10** agrees with the Cotton effects observed by Kabat *et al.*[2].

The assignment of bands to specific chromophores may be based on the c.d. curves of **7**, of its methyl β-D-ketoside (**8**), and of **9**, which contain only the amide chromophore. These compounds exhibit a single positive c.d. band, **7** and **8** at λ 192 nm and **9** at λ 194 nm. Consequently, in the spectra of **1** and its derivatives **2**–**6**, the intense, positive ellipticity band centered near λ 195 nm is probably associated with the *N*-acetyl chromophore and the weak band between λ 205 and 255 nm with the carboxyl chromophore.

If one assumes a $^2C_5$ conformation for the α- and β-D-ketosides*, the orientation of the carboxyl group at C-2 is axial in the former and equatorial in the latter compounds. The carboxyl group band (λ 205 and 255 nm) should, therefore, reflect the different steric position of this chromophore group. In fact, comparison of the curves of α-D- and β-D-ketosides (see Fig. 1) suggests that a negative sign indicates a carboxyl group in axial (α-D-ketosides) orientation and a positive sign in equatorial orientation (β-D-ketosides). This result is similar to that of other monosaccharides where similar change in orientation of the C-2 as well as C-4 chromophores results in a change of optical properties[8,9]. Thus, the anomeric configuration of **1** in various compounds may be ascertained by the Cotton effects between λ 205 and 255 nm.

The Cotton effect observed for the carboxyl group (λ 205–255 nm) approximately corresponds to that of the carboxyl group of uronic acids[3] (between λ 206 and 235 nm) and of acyclic acids[9] (λ 210–246 nm). The Cotton effect observed for the *N*-acetyl group (λ_{max} ~195 nm) differs, however, from that shown by 2-acetamido-2-deoxy sugars and by blood-group substances containing only the acetamido chromophore,

*The $^2C_5$ conformation for **1**, its methyl ester, and the corresponding *O*-acetyl and *O*-methyl derivatives was established by n.m.r. spectroscopy[7].

as well as by gangliosides and glycosaminoglycans having both the acetamido and the carboxyl chromophore[1,2,4] (λ_{max} 210–215 nm). One may conclude that the acetamido band of **1** and its derivatives studied so far is shifted to shorter wavelengths. It should be emphasized, however, that the observed position of the peaks in the spectra of **1–6** and **10** may not actually define the transition wavelength because of the overlap of the contributions of the acetamido and carboxyl groups. Nevertheless, the difference in the c.d. spectra between **1** and its derivatives on the one hand and 2-acetamido-2-deoxy sugars on the other hand may be useful in the study of the structure of complex biological substances containing both types of sugar components*.

The c.d. data presently available on the α-D-ketosides **2–4** and on the trisaccharide **10** indicate that the position of the negative band corresponding to the carboxyl group is shifted to shorter wavelengths and, furthermore, that the magnitude of the ellipticity increases with the size of the aglycon group (see Table I). No negative c.d. band but only a trough is discernible in the spectrum of the methyl α-D-ketoside (**2**), whereas the ethyl α-D-ketoside (**3**) shows a dichroic absorption of $\Delta\varepsilon$ −0.035, and the allyl α-D-ketoside of $\Delta\varepsilon$ −0.100; the spectrum of **10** shows the most negative band with $\Delta\varepsilon$ −0.450. Since these aglycon groups do not show any absorption band in this spectral region, the observed effects cannot result from a superimposition of their Cotton effects on that of the carboxyl group, but are rather due to inherent stereochemical factors.

It has been previously reported[4,10] that the preferred orientation of a hydroxymethyl or carboxyl group at C-5 of a pyranoside ring depends on the steric arrangement of neighboring groups, and that the contribution of optically active chromophores in monosaccharide derivatives is due to stereochemical parameters. Structural parameters of the aglycon residues may, in an analogous manner, influence the preferred alignment of the carboxyl group at C-2 and its interaction with other functional groups. Consequently, the c.d. band contributed by the carboxyl group may also be influenced by the structure of the aglycon. Although the nature of these stereochemical effects is at present difficult to determine, one may conclude that the various intensities of the contribution of the carboxyl group in **2–4** and **10** is the result of those parameters. Since only the c.d. spectra of the methyl β-D-ketosides **5** and **6** was studied, an influence of the structural parameters of the aglycon on the c.d. spectrum of β-D-ketosides cannot be discussed yet.

Although the present results are valuable in determining the structural parameters of *N*-acetylneuraminic acid derivatives, further studies on the assignment of particular ellipticity bands to particular chromophore transitions must be performed with derivatives containing various substituents.

*Complex c.d. curves showing four major bands between λ 185 and 250 nm have been described for some gangliosides[1-6]. Based on the results obtained with compounds **1–10**, both the negative band at λ ~220 nm and the positive one at λ ~187 nm are contributions of the *N*-acetylneuraminic acid residue, whereas the negative bands at λ ~210 nm and λ 196–202 nm are probably contributed by the 2-acetamido-2-deoxy sugars. The sign and position of the Cotton effect at λ ~220 is compatible with the well known α-D-ketosidic linkage of *N*-acetyl-D-neuraminic acid in gangliosides.

EXPERIMENTAL

Materials. — The methyl (**2**), ethyl (**3**), and allyl (**4**) glycosides of *N*-acetyl-α-D-neuraminic acid were synthesized by a modified Koenigs–Knorr reaction[11]. The synthesis of **7**, **8**, and **9** was described previously[12], and that of **5** and **6** performed according to Kuhn *et al.*[13]. *N*-Acetylneuraminic acid (**1**) and the trisaccharide **10** were isolated from meconium and bovine colostrum, respectively. The purity of all substances was controlled by elemental analyses, optical rotation, and chromatography. Compounds **2–4** were completely split by *N*-acylneuraminate hydrolase (neuraminidase, EC 3.2.1.18) from *Vibrio cholerae.*

Method. — The c.d. spectra were recorded with a Roussel–Jouan Circular Dichrograph II (Roussel-Jouan et Co., Paris, France). The concentration of the aqueous solution, pH 3.0–3.5, was 0.2–1%. Quartz cells with path–lengths of 0.02, 0.05, 0.1, 0.2, and 0.5 cm were used. The estimated error for c.d. measurements is ±3% above λ 200 nm and up to 10% below λ 200 nm. The coefficient of dichroic absorption $\Delta\varepsilon$ is expressed by $\Delta D/c \cdot l$. ΔD is the difference in the observed values of absorbance between left and right circularly-polarized light, c is the molar concentration, and l is the path–length in cm.

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