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STUDIES ON SIALIC ACIDS I. DETERMINATION OF ANOMERIC CONFIGURATION OF NEURAMINIC ACID DERIVATIVES BY CIRCULAR DICHROISM1)

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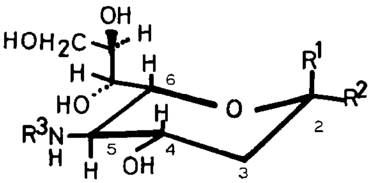
Summary: CD spectra were recorded for methyl α- and β-glycosides of D-neuraminic acid, and the band at the wave-length lower than 200 nm was attributed to the acetamido group. The Cotton effect at higher wave-length around 220 nm arose from the $n-\pi^*$ transition of the carboxyl group. Thus α-linked glycosides showed a negative band, while β-glycosides gave arise to a positive band.

N-Acetyl-D-neuraminic acid (5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosidic acid; 1) is widely distributed in the implication of membrane glycoproteins in animal cells. From the determination of the crystalline structure of the compound by X-ray analysis2) and NMR studies,3,4) it has been shown that the conformation of the pyranose ring of 1 in the natural products is 1C and the configuration at C-2 is α.5)

In this paper we wish to report the synthesis of methyl α- and β-glycosides of N-acetylneuraminic acid and some related derivatives, and to confirm their stereochemistry by means of NMR and CD studies.

N-Acetyl-D-neuraminic acid (1) was obtained from edible birds' nest substance in high yield (6.6%) by the method of Czarniecki and Thoruton.4) Methyl β-glycoside of methyl 5-N-acetyl-D-neuraminate (2),6) methyl α-glycoside of methyl 5-N-acetyl-D-neuraminate (4),7,8) methyl β-glycoside of D-neuraminic acid (5),9) and methyl 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranoside (6)10) were prepared following the procedures shown in the above literatures.

Table I. Derivatives of N-Acetyl-D-neuraminic Acid



Compound	R^1	R^2	R^3
1	OH	COOH	Ac
2	OMe	COOMe	Ac
4	COOMe	OMe	Ac
5	OMe	COOH	H
6	OMe	CH_2OH	Ac

Methyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranoside (**7**) was prepared from methyl α-glycoside of methyl 5-*N*-acetyl-4,7,8,9-tetra-*O*-acetyl-D-neuraminate (**3**)8) with sodium methoxide to yield **4**. This compound (**4**) was reduced with $NaBH_4$ in methanol and the reaction mixture was treated with Dowex-50 (H^+ type) at -20° to yield 40% of **7** as colorless needles, mp 162-165°.11)

Methyl α-glycoside of D-neuraminic acid (**8**) was prepared by treatment of **3** with PCl_5, subsequently, CH_3OH, and $Ba(OH)_2$.12) The reaction mixture was treated with Dowex-2 (OH^- type) to yield 12% of **8** as a white powder.11)

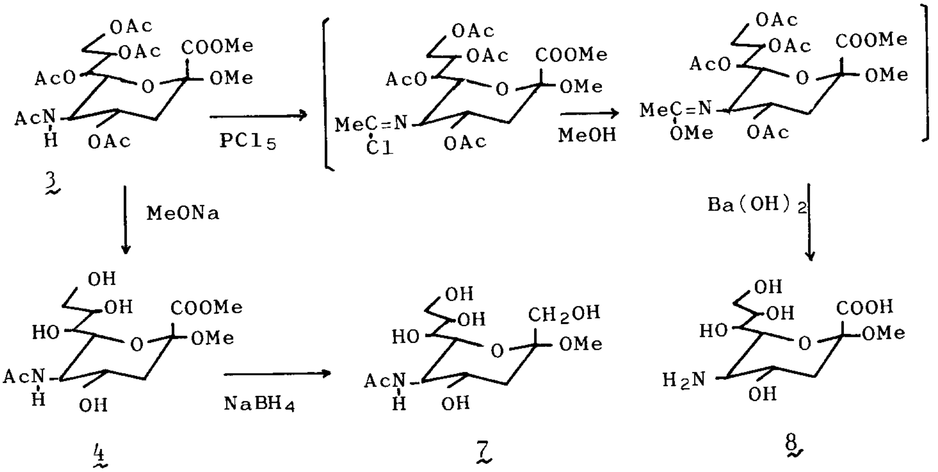


Figure 1 shows the CD spectra of two typical β- and α-methyl glycosides (**2**, **4**). The large positive band at low wave-length at 194 nm was attributed to the acetamido group, while the smaller spectral features around 210-225 nm were assigned to the carboxyl chromophore.

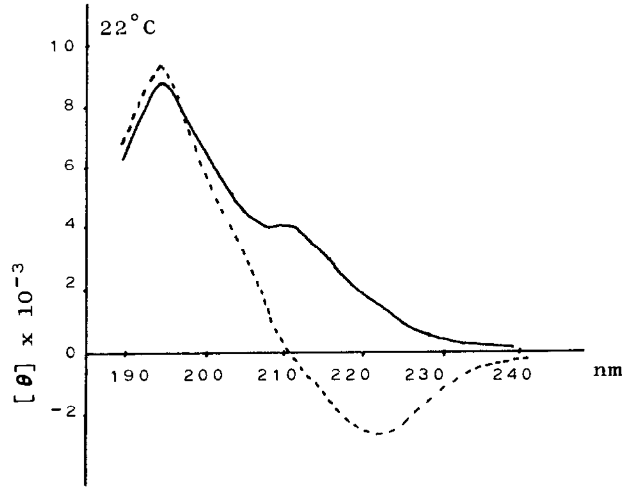


Figure 1. CD curves of **2** (———) and **4** (----) in H_2O

This was confirmed from CD data of the corresponding carboxy-reduced compound (6, 7) summarized in Table II, in which the band at higher wave-length was lost. On the other hand, methyl β- and α-glycosides of D-neuraminic acid (5, 8) showed that the band at lower wave-length was lost.

Table II. CD Data of the Typical α- and β-Glycosides (H_2O, 22°C)

Compound (Anomeric configuration)	Lower band (nm)	Higher band (nm)	Molecular ellipticity [θ]
5 (β)	-	215	+2040
6 (β)	193	-	+12600
7 (α)	193	-	+12730
8 (α)	-	216	-1520

In conclusion, the strong band at lower wave-length is attributed to the acetyl group and the sign of the Cotton effect is positive in both α- and β-anomers. In contrast, the sign of the $n-\pi^*$ Cotton effect at higher wave-length is positive for β-glycosides (1, 2, 5) and negative for α-glycosides (3, 4, 8).

Figure 2 shows spectral changes with time which were observed for methyl β- and α-glycosides (2, 4). β-Form compound (2) in 1N HCl (methanol) shows a positive $n-\pi^*$ Cotton effect at 222 nm ([θ] +2432) owing to the carboxyl group, and the figure of CD curve did not change after 20 hr. In contrast, α-form compound (4) in 1N HCl (methanol) shows a negative $n-\pi^*$ Cotton effect at 213 nm ([θ] -4174) owing to the carboxyl group, and the figure of CD curve changed

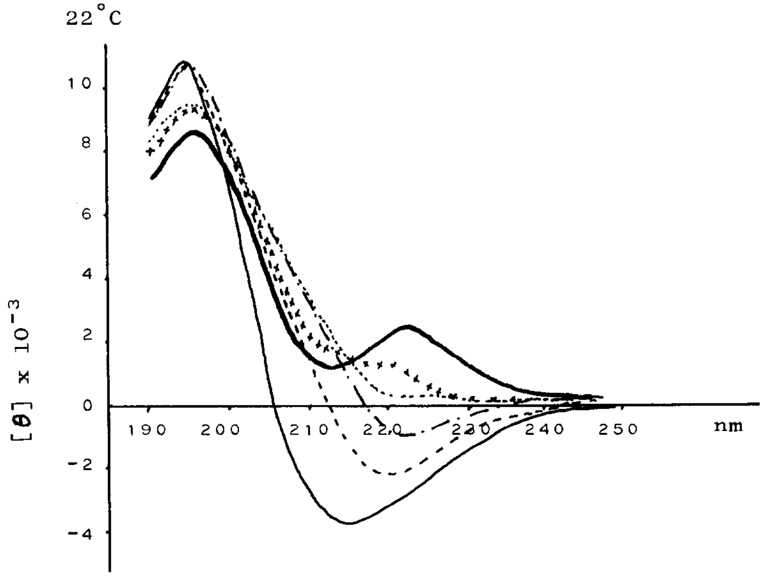


Figure 2. CD curves of 2 (——) and 4 (—— ; 2 hr--- ; 4 hr -—; 8 hr..... ; 20 hr***)

by the elapse of time as shown in Figure 2. After 20 hr, the carboxyl negative band changed to positive and the curve approached that of β-glycoside (2) (70% of β-glycoside and 20% of α-glycoside were observed by the NMR spectrum)[13].

This evidence probably supports that the bonded N-acetyl-D-neuraminic acid in animal cells is α-glycoside, while the extracted N-acetyl-D-neuraminic acid is β-glycoside by the configurational change.

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REFERENCES

1) A part of this work was presented at the 23rd Symposium of the Chemistry of Natural Products, p. 295 (1980).
2) A. M. O'Connel, Acta Cryst., B29, 2320 (1973); J. L. Flippen, Acta Cryst., B29, 1881 (1973); A. Biedl, Naturwissenshaften, 58, 95 (1971).
3) P. Lutz, W. Lochinger, and G. Taigel, Chem. Ber., 101, 1089 (1968); U. Dabrowski, H. Friebolin, R. Brossmer, and M. Supp, Tetrahedron Lett., 4637 (1979).
4) M. F. Czarniecki and E. R. Thornton, J. Am. Chem. Soc., 99, 8273 (1977).
5) A. Rosenberg and C. -L. Schengrund, Ed., "Biological Roles of Sialic Acid", Plenum Press, New York, N. Y., 1976; R. Schauer, Angew. Chem. Internat., 12, 127 (1973).
6) R. K. Yu and R. Ledeen, J. Biol. Chem., 244, 1306 (1969).
7) P. Meindl and H. Tuppy, Monats. Chem., 96, 802 (1965).
8) R. Kuhn, P. Lutz, and D. L. MacDonald, Chem. Ber., 99, 611 (1966).
9) R. Scheuer and H. -P. Buscher, Biochim. Biophys. Acta, 338, 369 (1974).
10) R. Brossmer and L. Holmouist, Z. Physiol. Chem., 352, 1715 (1971).
11) All new compounds show satisfactory elemental analyses and spectral properties.
^{1}H-NMR [60 MHz, D_2O, δ ppm (DSS)]
7: 1.73 (1H, dd, J=11.0 and 13.0 Hz, 3-Hax), 2.21 (3H, s, $NHCOCH_3$), 2.29 (1H, dd, J=4.5 and 13.0 Hz, 3-Heq), 3.32 (3H, s, OCH_3).
8: 1.70 (1H, dd, J=4.6 and 13.0 Hz, 3-Hax), 2.55 (1H, dd, J=11.5 and 13.0 Hz, 3-Heq), 3.40 (3H, s, OCH_3).
12) A. Koda, K. Takanobu, I. Isaka, T. Kashiwagi, K. Takahashi, S. Kawahara, and M. Murakami, YAKUGAKU ZASSHI, 92, 459 (1972).
13) Isosbestic point is not observed in Figure 2, probably owing to form about 10% of unknown compound from the NMR spectrum.

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