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Studies on Sialic Acids. VII. The Crystal and Molecular Structure of *N*-Acetyl-2,3-dehydro-2-deoxyneuraminic Acid

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The structure of *N*-acetyl-2,3-dehydro-2-deoxyneuraminic acid has been established by means of X-ray crystal structure analysis. The ring system was determined to have a $^4H_5$(D) conformation.

Keywords——sialic acid; X-ray analysis; torsional angle; stereochemistry; hydrogen bond; neuraminic acid

The sialic acid, *N*-acetyl-2,3-dehydro-2-deoxyneuraminic acid (5-acetamido-2,6-anhydro-2,3,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid, Neu2en5Ac), is widely distributed in nature.[1)]

It lacks the glycosidic hydroxy group in *N*-acetylneuraminic acid (Neu5Ac), but has significant biological activities.[2,3)]

The present paper describes the determination of the molecular dimensions, stereochemistry and intermolecular packing of Neu2en5Ac. A great deal of interest has centered on the conformation of Neu2en5Ac since its stereochemistry may well affect its activity. Already, the crystal structures of several sialic acids, β-D-*N*-acetylneuraminic acid dihydrate,[4)] β-D-*N*-acetylneuraminic acid methyl ester monohydrate,[5)] methyl β-glycosidic neuraminic acid,[6)] and the 1,4-lactone derivative of *N*-acetylneuraminic acid,[7)] have been established by X-ray analysis.

Experimental

Preparation of Neu2en5Ac——A solution of 0.02 g of concentrated sulfuric acid in acetic anhydride (1 ml) was slowly added to a solution of Neu5Ac methyl ester (1.0 g, 3 mmol) in 10 ml of acetic anhydride. After being stirred for 10 h at room temperature, the reaction mixture was poured into ice-water. The mixture was stirred, saturated with sodium hydrogen carbonate, and then extracted with chloroform (3 × 100 ml). The chloroform layer was evaporated to give a syrup, which was purified by recrystallization from ethanol to give Neu2en4,5,7,8,9Ac$_5$ (1.3 g, 90%) as needles.[8)] mp 132—135 °C. $[\alpha]_D^{20}$ +40° (c=1, MeOH). *Anal.* Calcd for $C_{20}H_{27}NO_{12}$: C; 50.74, H; 5.75, N; 2.96. Found: C; 50.72, H, 5.80, N; 2.95.

A solution of 473 mg of Neu2en4,5,7,8,9Ac$_5$ in 2 ml of 1 N NaOH was stirred for 4 h at room temperature. The solution was diluted with water, deionized on Dowex-50 (H^+) resin, and evaporated. The residue was purified by recrystallization from methanol to give Neu2en5Ac monohydrate (250 mg, 86%) as fine needles. mp 148—149 °C (dec.). $[\alpha]_D^{20}$ +48° (c=1, H_2O). (Ref. mp 137—140 °C, $[\alpha]_D^{20}$ +41.6° (c=0.25, H_2O)).[9)] *Anal.* Calcd for $C_{11}H_{19}NO_9$: C; 42.72, H; 6.19, N; 4.53. Found: C; 42.70, H; 6.15, N; 4.53.

Neu2en5Ac was obtained as colorless prisms by recrystallization from methanol–ether. mp 227—228 °C (dec.). $[\alpha]_D^{20}$ +51° (c=1, MeOH). *Anal.* Calcd for $C_{11}H_{17}NO_8$: C; 45.36, H; 5.88, N; 4.81. Found: C; 45.35, H; 5.90, N; 4.78.

A crystal of Neu2en5Ac with the dimensions of 0.45 × 0.25 × 0.25 mm was used for the intensity measurements. The density was measured by the flotation method in a mixture of petroleum ether and carbon tetrachoride. The cell constants were determined by the least-squares procedure from the 2θ values of 24 reflections measured on a diffractometer using monochromated Cu$K\alpha$ radiation. Three-dimensional intensity data were collected on Rigaku

TABLE I. Atomic Coordinates (10^4) and Their Standard Deviations in Parentheses and Equivalent Isotropic Temperature Factors for Non-hydrogen Atoms

Atom	x	y	z
O(1)	3133 (4)	256 (2)	7488 (6)
O(2)	3862 (4)	230 (2)	5132 (8)
O(3)	2803 (4)	500 (2)	3524 (7)
O(4)	339 (4)	212 (2)	5716 (8)
O(5)	1361 (5)	−269 (2)	9712 (9)
O(6)	3568 (4)	−121 (1)	9652 (7)
O(7)	3939 (4)	506 (1)	11806 (7)
O(8)	5779 (4)	286 (2)	12831 (7)
N	1344 (5)	169 (2)	9380 (8)
C(1)	3170 (6)	356 (2)	4843 (10)
C(2)	2614 (6)	348 (2)	5972 (9)
C(3)	1724 (6)	393 (2)	5521 (10)
C(4)	1188 (5)	342 (2)	6645 (10)
C(5)	1737 (5)	164 (2)	8044 (9)
C(6)	2703 (5)	271 (2)	8720 (9)
C(7)	3353 (5)	123 (2)	10169 (10)
C(8)	4205 (5)	287 (2)	11041 (9)
C(9)	4946 (5)	143 (2)	12355 (9)
C(10)	1228 (6)	−49 (2)	10143 (10)
C(11)	935 (7)	−6 (3)	11628 (12)

TABLE II. Fractional Coordinates (10^3) and Equivalent Isotropic Temperature Factors for Hydrogen Atoms

Atom	x	y	z	B
H(O3)	298 (6)	710 (23)	319 (11)	7.3
H(C3)	135 (6)	504 (23)	431 (11)	2.7
H(C4)	101 (5)	522 (20)	704 (9)	1.2
H(O4)	−26 (6)	346 (24)	480 (12)	4.8
H(C5)	177 (7)	−67 (26)	766 (13)	5.4
H(C6)	268 (6)	492 (23)	913 (11)	3.1
H(C7)	305 (6)	58 (24)	1108 (12)	2.2
H(C8)	446 (7)	359 (27)	1020 (13)	3.3
H(C9)	497 (6)	−32 (22)	1192 (11)	2.9
H(C9)	472 (6)	117 (23)	1337 (11)	3.6
H(O8)	577 (7)	411 (28)	1310 (14)	4.5
H(N)	118 (7)	380 (28)	972 (14)	5.3

automatic four-circle diffractometer (AFC-5) with graphite-monochromated Cu$K\alpha$ radiation. Three standard reflections were measured every 50 reflections during the course of collection. The maximum deviation of the standards from their mean values was 0.035. In total, 1382 independent reflections were collected. Reflections having an intensity exceeding the corresponding standard deviations by $|F_o| > 3\sigma(|F_o|)$ were treated as observed, and 1175 reflections with $2\theta \leqq 135°$ were retained and corrected for Lorentz and polarization factors, but not for absorption.

Crystal Data——$C_{11}H_{17}NO_8$ M_r 291.26, crystal system monoclinic, space group $p2_1$, $a=15.623(5)$Å, $b=5.155(2)$, $c=8.648(3)$, $\beta=109.91(3)°$, $V=654.9(4)$Å^3, $Z=2$, $D_c=1.476\,g\cdot cm^{-3}$, $D_o=1.48\,g\cdot cm^{-3}$.

Determination and Refinement of the Structure——The crystal structure was solved by the multi-solution method (MULTAN),[10] using 125 normalized structure factors with $E \geqq 1.3$. Origin and enantiomorph fixings were carried out automatically by the program. Only one of sixteen possible solutions gave high combined figures of merit and the E map showed reasonable positions and bond relations for all the non-hydrogen atoms. Refinement of the positional parameters of the twenty atoms was carried out by a least-squares diagonal-matrix method, the quantity minimized being $\Sigma\omega\,(|F_o| - |F_c|)^2$, with $\omega=1.0$ for all the reflections used. Five cycles of calculation gave an R-value of 0.19.

TABLE III. Anisotropic Thermal Parameters (10^4) for Non-hydrogen Atoms

Atom	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
O(1)	13 (2)	291 (25)	46 (6)	11 (7)	9 (3)	44 (12)
O(2)	25 (3)	344 (29)	96 (8)	37 (8)	28 (4)	71 (14)
O(3)	32 (3)	380 (30)	75 (8)	39 (8)	26 (4)	95 (14)
O(4)	13 (2)	302 (28)	109 (9)	−11 (7)	5 (4)	−3 (14)
O(5)	57 (4)	154 (23)	141 (10)	−10 (8)	60 (5)	−12 (14)
O(6)	29 (3)	132 (20)	69 (7)	10 (6)	19 (4)	15 (11)
O(7)	29 (3)	145 (21)	58 (7)	4 (6)	17 (4)	8 (11)
O(8)	16 (2)	255 (26)	97 (8)	−13 (7)	11 (4)	−42 (13)
N	17 (3)	189 (27)	70 (9)	−4 (8)	25 (4)	7 (14)
C(1)	26 (4)	227 (33)	52 (10)	−9 (10)	15 (5)	−1 (17)
C(2)	18 (3)	225 (32)	44 (9)	19 (9)	11 (5)	24 (16)
C(3)	16 (3)	260 (37)	56 (10)	5 (9)	4 (5)	6 (17)
C(4)	11 (3)	212 (31)	56 (10)	−7 (9)	7 (4)	−7 (16)
C(5)	13 (3)	161 (30)	62 (9)	−5 (8)	16 (4)	−20 (16)
C(6)	14 (3)	126 (27)	43 (9)	4 (8)	11 (4)	20 (14)
C(7)	15 (3)	135 (29)	56 (10)	−10 (8)	7 (5)	16 (15)
C(8)	16 (3)	121 (29)	47 (9)	−6 (8)	7 (4)	17 (14)
C(9)	13 (3)	187 (31)	51 (9)	−2 (8)	−1 (4)	13 (16)
C(10)	22 (4)	217 (34)	77 (11)	−11 (9)	23 (5)	1 (18)
C(11)	46 (5)	396 (48)	100 (13)	−12 (14)	49 (7)	−7 (24)

TABLE IV. Bond Lengths (l/Å) and Their Standard Deviations in Parentheses

O(1)–C(2)	1.37 (1)	O(1)–C(6)	1.44 (1)
O(2)–C(1)	1.21 (1)	O(3)–C(1)	1.32 (1)
O(4)–C(4)	1.46 (1)	O(5)–C(10)	1.23 (1)
O(6)–C(7)	1.41 (1)	O(7)–C(8)	1.44 (1)
O(8)–C(9)	1.43 (1)	N–C(5)	1.48 (1)
N–C(10)	1.35 (1)	C(1)–C(2)	1.51 (1)
C(2)–C(3)	1.33 (1)	C(3)–C(4)	1.51 (1)
C(4)–C(5)	1.53 (1)	C(5)–C(6)	1.53 (1)
C(6)–C(7)	1.52 (1)	C(7)–C(8)	1.54 (1)
C(8)–C(9)	1.51 (1)	C(10)–C(11)	1.52 (2)

TABLE V. Bond Angles (ϕ/°) and Their Standard Deviations in Parentheses

C(2)–O(1)–C(6)	114.5 (7)	C(5)–N–C(10)	121.7 (7)
O(2)–C(1)–O(3)	124.7 (9)	O(2)–C(1)–C(2)	121.5 (8)
O(3)–C(1)–C(2)	113.8 (8)	O(1)–C(2)–C(1)	110.2 (7)
O(1)–C(2)–C(3)	124.0 (8)	C(1)–C(2)–C(3)	125.3 (8)
O(4)–C(4)–C(3)	109.0 (7)	O(4)–C(4)–C(5)	108.5 (7)
C(3)–C(4)–C(5)	109.4 (7)	N–C(5)–C(4)	109.7 (7)
N–C(5)–C(6)	108.2 (7)	C(4)–C(5)–C(6)	107.4 (7)
O(1)–C(6)–C(5)	110.4 (6)	O(1)–C(6)–C(7)	103.4 (6)
C(5)–C(6)–C(7)	115.5 (7)	O(6)–C(7)–C(6)	110.6 (6)
O(6)–C(7)–C(8)	112.6 (6)	C(6)–C(7)–C(8)	110.5 (6)
O(7)–C(8)–C(7)	108.5 (6)	O(7)–C(8)–C(9)	107.6 (6)
C(7)–C(8)–C(9)	114.3 (7)	O(8)–C(9)–C(8)	110.7 (7)
O(5)–C(10)–N	123.9 (8)	O(5)–C(10)–C(11)	121.1 (9)
N–C(10)–C(11)	115.1 (8)		

The oxygen atoms and nitrogen atom were assigned on the basis of chemical considerations. Further refinement carried out with anisotropic thermal parameters gave an R-value of 0.09. All the hydrogen atoms except those of the methyl group and the hydroxy groups on O(6) and O(7) were found from difference maps, and they were refined with

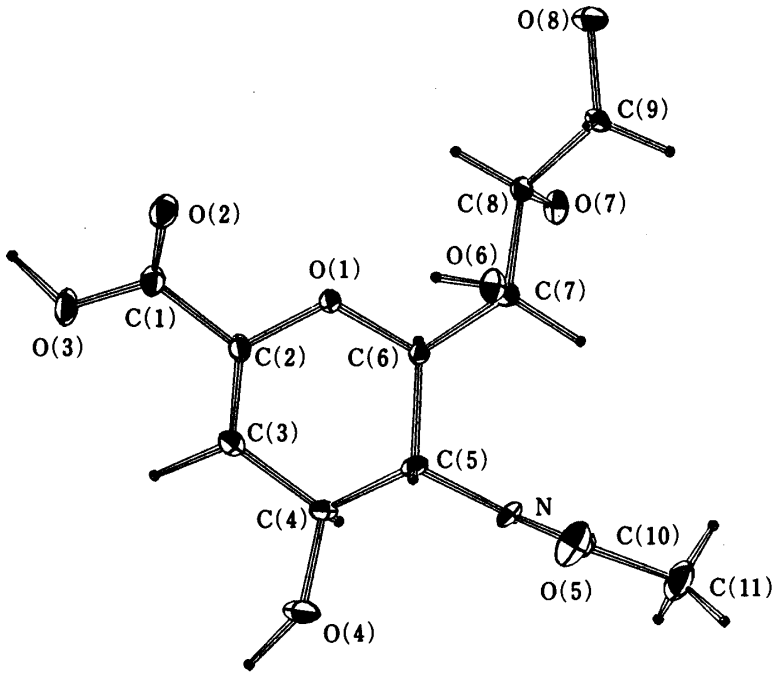

Fig. 1. A Perspective View of the Neu2en5Ac Molecule along the c Axis

TABLE VI. Some Torsional Angles (ϕ/°)

C(6)–O(1)–C(2)–C(1)	−172.9	O(1)–C(6)–C(7)–C(8)	−75.1
C(6)–O(1)–C(2)–C(3)	14.9	C(5)–C(6)–C(7)–C(8)	164.1
C(2)–O(1)–C(6)–C(5)	−47.9	C(6)–C(7)–C(8)–C(9)	172.5
C(2)–O(1)–C(6)–C(7)	−172.0	O(2)–C(1)–C(2)–O(1)	−18.6
O(1)–C(2)–C(3)–C(4)	0.8	O(2)–C(1)–C(2)–C(3)	153.4
C(1)–C(2)–C(3)–C(4)	−170.2	O(3)–C(1)–C(2)–O(1)	164.0
C(2)–C(3)–C(4)–O(4)	135.6	O(3)–C(1)–C(2)–C(3)	−24.0
C(2)–C(3)–C(4)–C(5)	17.2	O(6)–C(7)–C(8)–O(7)	168.3
C(3)–C(4)–C(5)–C(6)	−47.2	O(7)–C(8)–C(9)–O(8)	72.8
C(4)–C(5)–C(6)–O(1)	64.7	O(4)–C(4)–C(5)–N	76.5
C(4)–C(5)–C(6)–C(7)	−178.4	N–C(5)–C(6)–C(7)	−60.0

isotropic thermal parameters. Five cycles of refinement by the least-squares block-diagonal matrix method gave the final R value of 0.065 for 1175 reflections. The final atomic parameters are given in Tables I—III. The atomic scattering factors for C O,N were taken from by Cromer and Mann,[11] and that for H from Stewart *et al.*[12]

Results and Discussion

The bond lengths and angles are given in Tables IV and V. No abnormal lengths or angles were found in structure. The molecular framework with the numbering of the atoms is shown in Fig. 1. The absolute configuration of the asymmetric centers agrees with that found for a related molecule, methyl β-glycosidic neuraminic acid.[6]

From this perspective view of the molecule, the ring conformation is a normal half chair, namely, $^4H_5$(D) and has dimensions that generally agree with those for other glycals. It was concluded that the side-chain groups have very little effect on the ring conformation.

As shown in Table VI the torsional angle of C(4)–C(3)–C(2)–O(1) is 0.8°, which means that atoms C(4), C(3), C(2), and O(1) are placed on the same plane. Torsional angles C(6)–O(1)–C(2)–C(3) and C(2)–C(3)–C(4)–C(5) are 14.9° and 17.2°, respectively. The orientations of the hydroxy groups on the glycerol group are anti with O(6)–C(7)–C(8)–O(7) = 168.3° and

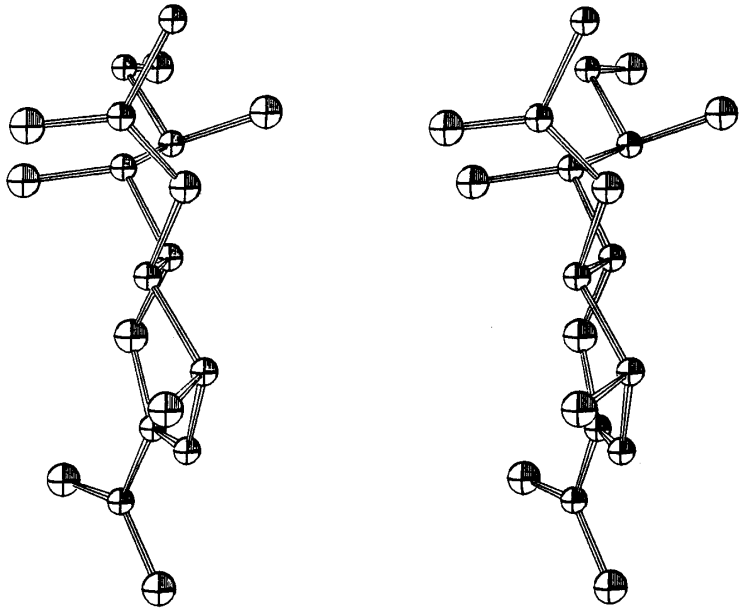

Fig. 2. Stereoscopic View of the Neu2en5Ac Molecule

TABLE VII. Bond Lengths (l/Å) of Intermolecular Hydrogen Bonds

O(2)–O(8)	2.82	O(3)–O(7)	2.67
O(4)–O(4)	2.90	O(5)–N	2.91
O(6)–O(7)	2.61	O(6)–O(8)	2.72

gauche with O(7)–C(8)–C(9)–O(8) = 72.8°, respectively. The *N*-acetyl group is *gauche* to both the hydroxy group on C(4) and the glycerol group on C(6). The crystal structure of the compound projected along the *a* axis is shown in Fig. 2, and the intermolecular hydrogen bonding distances are listed in Table VII.

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