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Studies on Sialic Acids. IX. Formation of a 1,7-Lactone Derivative by Direct Acetylation of *N*-Acetylneuraminic Acid

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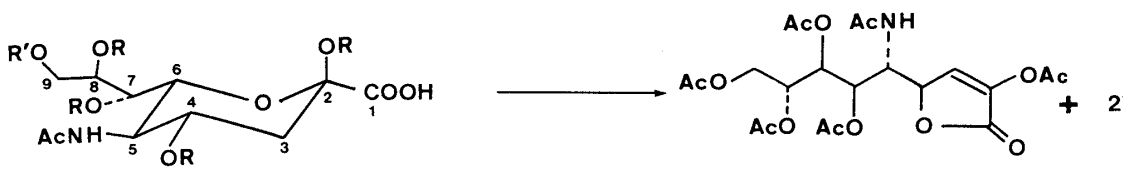
A new lactone compound was isolated as a minor by-product from the reaction mixture of *N*-acetylneuraminic acid with acetic anhydride in pyridine, in addition to the major product, 2,4,7,8,9-penta-*O*-acetyl-*N*-acetylneuraminic acid. The structure of the new compound was elucidated to be 5-acetamido-2,4,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosono-1,7-lactone by nuclear magnetic resonance, mass spectroscopy and X-ray crystal analysis. It was revealed that the absolute configurations of the asymmetric centers of the new compound were consistent with those for the original *N*-acetylneuraminic acid with the conversion of the pyranose ring conformation from $^{2}C_{5}$(D) to $^{5}C_{2}$(D).

Keywords——*N*-acetylneuraminic acid; lactone; X-ray crystal analysis; acetylation; ^{1}H-NMR; sialic acid

We have been investigating various *O*-acylated derivatives of neuraminic acid (**1**) because of their recently reported biological implications, such as the significant effects on enzyme functions, complement activation and antigenicity,[1-3] and the recognition of 5-acetamido-9-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid (**3**) as an essential determinant of the cell surface receptors of influenza A and B viruses.[4]

In a previous paper,[5] we described the formation of 1,4-lactone derivatives of *N*-acetylneuraminic acid under the acetylation conditions which we employed. In this report, we describe the isolation of a minor product from the reaction mixture and the determination of its structure by means of mass and proton nuclear magnetic resonance (^{1}H-NMR) spectrometry and X-ray crystal analysis.

The direct acetylation of **1** had been studied by Meindl *et al.*[6] and Korlin *et al.*,[7] and 2,4,7,8,9-penta-*O*-acetyl-*N*-acetylneuraminic acid (**2**)[6] and 5-acetamido-2,6,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-noneno-1,4-lactone (**4**)[7] were reported as the products. The structure of compound **4** was proposed to be as shown in Chart 1, but the infrared (IR) and ultraviolet (UV) spectral data did not fully support this.



1 : R = H , R' = H
2 : R = R' = Ac
3 : R = H , R' = Ac

4

Chart 1

Therefore, we reexamined the products of acetylation of **1** performed according to Korlin *et al.*[7] with acetic anhydride in pyridine overnight at room temperature. From the reaction mixture, the acidic compound **2** was isolated as the major product in 90% yield and a neutral compound **5** as the minor by-product in 6% yield, but compound **4** could not be isolated. Compound **5** was purified by recrystallization from ethanol to give colorless prisms, mp 207—209 °C, $[\alpha]_D^{20}+71.4°$ ($c=1$, MeOH). This was an unknown compound having a UV absorption maximum at 204 nm, and was different from the previously reported 1,4-lactone derivative **4** in various physical constants. Compound **5** did not give a positive qualitative reaction for carboxyl group, had a molecular formula of $C_{19}H_{25}NO_{12}$, determined by means of elementary analysis and mass spectroscopy, and showed five singlet signals between δ 2.06 to 2.12 ppm in the 500 MHz ^{1}H-NMR spectrum (Table I), indicating the presence of a methyl moiety of an *N*-acetyl group and four newly induced *O*-acetyl groups. In the IR spectrum, compound **5** showed the C=O stretching band at 1770 cm^{-1}, which can be assigned to a γ-lactone.

The possible sites of the lactone formation were assumed to be between the carboxyl group at position 2 and the hydroxyl group at position 4,7,8, or 9. Generally, the mass spectrum of a lactone compound exhibits an ion peak caused by α-breakage of the ether moiety as a characteristic fragment ion. A precise examination of the mass spectrum detected a peak at *m/z* 314 which corresponded to $[M-(CH_3COOCHCH_2OCOCH_3)]^+$, as shown in Fig. 1.

All the signals in the ^{1}H-NMR spectrum of **5** were assigned by means of homonuclear double-resonance analysis and the chemical shifts and coupling constants were determined as

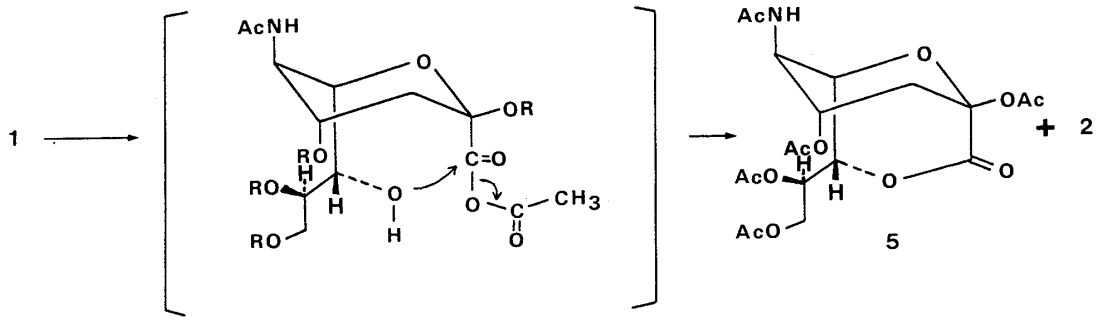


Chart 2

TABLE I. ^{1}H-NMR Spectral Data of Compound **2** in D_2O (TSP) and of Compound **5** in $CDCl_3$ (TMS)[a]

Proton	**2** Multiplicity (*J*, Hz)	**5** Multiplicity (*J*, Hz)
3-H	1.87 dd (3H,3H′=13.6, 3H,4H=11.4)	2.15 dd (3H,3H′=14.5, 3H,4H=3.8)
3-H′	2.46 dd (3H′,3H=13.6, 3H′,4H=5.1)	2.37 dd (3H′,3H=14.5, 3H′,4H=0—1)
4-H	5.28 ddd (4H,3H=11.4, 4H,3H′=5.1, 4H,5H=10.3)	5.11 ddd (4H,3H=3.8, 4H,3H′=0—1, 4H,5H=0—1)
5-H	3.94 (5H,4H=10.3, 5H,6H=10.6)	4.23 dd (5H,4H=0—1, 5H,NH=8.4)
6-H	4.10 dd (6H,5H=10.6, 6H,7H=1.8)	4.19 s
7-H	5.43 dd (7H,6H=1.8, 7H,8H=7.7)	4.64 d (7H,8H=8.4)
8-H	5.17 ddd (8H,7H=7.7, 8H,9H=4.0, 8H,9H′=2.9)	5.47 ddd (8H,7H=8.4, 8H,9H=4.4, 8H,9H′=2.4)
9-H	4.21 dd (9H,8H=4.0, 9H,9H′=12.8)	4.29 dd (9H,8H=4.4, 9H,9H′=12.7)
9-H′	4.45 dd (9H′,8H=2.9, 9H′,9H=12.8)	4.47 dd (9H′,8H=2.4, 9H′,9H=12.7)
Others	1.94 s, 2.04 s, 2.08 s 2, 2.17 s 2	2.06 s, 2.07 s, 2.10 s, 2.11 s, 2.12 s

a) Spectra measured at 500 MHz.

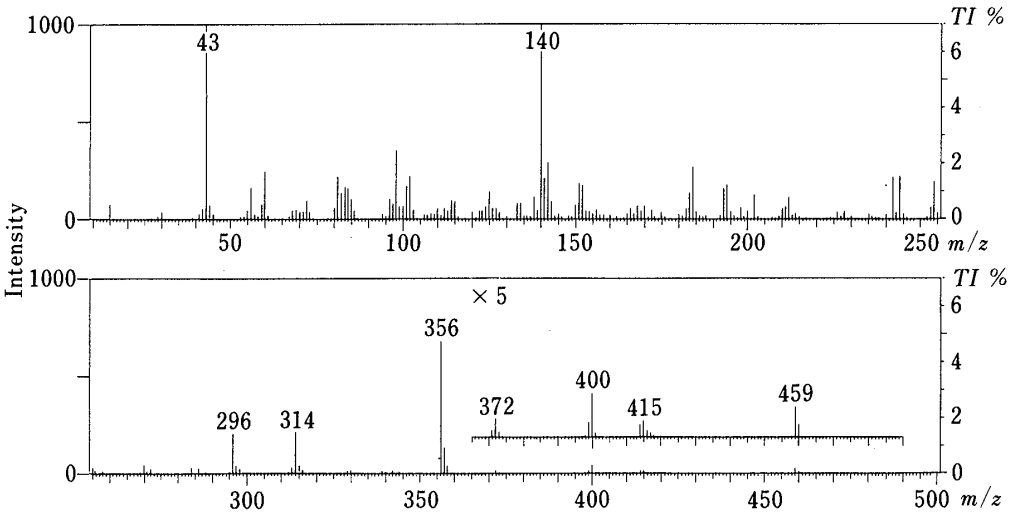


Fig. 1. The EI Mass Spectrum of **5**

shown in Table I.

The structure of **5** was deduced from a comparison of the spectral data of **2** and **5**. The ^{1}H-NMR spectra showed the signal due to 7-H at δ 4.64 ppm for **5** and at δ5.43 ppm for **2**.

Furthermore, the vicinal J values on the pyranose ring in **5** were all small (1—4 Hz), reflecting the structural change in **1**, that is, the conversion of the pyranose ring in **5** from $^2C_5$ (D) to $^5C_2$ (D). These results indicated that the lactone linkage was formed between the C(2)-carboxyl and C(7)-hydroxyl groups, and compound **5** was identified as 5-acetamido-2,4,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosono-1,7-lactone.

In order to confirm the stereochemistry of **5**, X-ray crystallography was conducted (see Fig. 2 and the experimental part). The vicinal coupling constants between the hydrogen atoms on the rings of **5**, shown in Table I, indicate that the ring has the same conformation both in the crystal and in solution.

As shown in Chart 2, the formation of the 1,7-lactone could be envisaged in terms of mixed anhydride formation between the carboxyl group of the *N*-acetylneuraminic acid and acetic anhydride followed by ring closure with the hydroxyl group at the most sterically reasonable position.

Experimental

Melting points were determined in a capillary tube, and are uncorrected. ^{1}H-NMR spectra were measured with a JEOL GX-500 spectrometer, and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were obtained at 125 MHz in a pulsed Fourier-transform mode on a JEOL GX-500 instrument with Me_4Si (TMS) as an internal standard in $CDCl_3$ and 3-(trimethylsilyl)propionic acid-d_4 sodium salt (TSP) in D_2O at room temperature. Electron impact (EI) mass spectra were obtained on a JEOL JMS-D300, and IR spectra on a JASCO IR-810 spectrometer. Thin layer chromatography (TLC) was conducted on precoated silica gel plates (Merck GF-254), and compounds were detected by UV fluorescence quenching and by spraying 5% sulfuric acid.

Reaction of *N*-Acetylneuraminic Acid (Neu5Ac) 1 with Acetic Anhydride——To a solution of **1** (0.54 g) in pyridine was added acetic anhydride (2.5 ml) at room temperature and the mixture was stirred at room temperature overnight. Ethanol (25 ml) was then added to the reaction mixture and the solvents were evaporated off *in vacuo*. After the addition of water to the residue, the insoluble material was extracted twice with chloroform (50 ml). The aqueous layer was treated with Dowex-50 (H^+) and filtered. The filtrate was lyophilized to give 2,4,7,8,9-penta-*O*-acetyl-*N*-acetylneuraminic acid (**2**, 0.95 g, 90%) as a white powder. mp 147 °C (dec.), $[\alpha]_D^{25}$ −11.2° (c=0.3, H_2O) (ref.[6]) mp 145 °C (dec.), $[\alpha]_D^{20}$ −10.3° (c=0.3, H_2O)). The combined chloroform extracts were dried over sodium sulfate, filtered and evaporated to dryness. The residue was purified by recrystallization from ethanol to give 50 mg (6%) of **5** as colorless prisms. mp. 207—209 °C, $[\alpha]_D^{23}$ +71.4° (c=1, MeOH). UV λ_{max}^{MeOH} nm(ε): 204 (1300). The mass spectrum chart is

shown in Fig. 2. *Anal.* Calcd for $C_{19}H_{25}NO_{12}$: C; 49.67, H; 5.49, N; 3.05. Found: C; 49.90, H; 5.47, N; 3.08. IR ν_{max}^{KBr} cm^{-1} : 1770, 1742, 1660. The ^{1}H-NMR data are given in Table I. ^{13}C-NMR ($CDCl_3$, TMS) δ: 170.6, 169.9, 169.5, 169.1, 167.9 and 164.5 (C=O×6), 91.2 (C-2), 61.5 (C-9), 33.1 (C-3).

Crystal Data for 5——A crystal with the dimensions of $0.3\times0.3\times0.2\,mm^3$ was used for the structure determination. The cell dimensions and diffraction intensities were measured on a Rigaku automatic four-circle diffractometer, using graphite-monochromated CuK_α radiation.

Crystal Data: $C_{19}H_{25}NO_{12}$, orthorhombic, space group $p2_12_12_1$, $a=15.426$ (2), $b=15.786$ (2), $c=9.235$ (2) Å, $z=4$, $D_c=1.375\,g\cdot cm^{-3}$, $D_o=1.35\,g\cdot cm^{-3}$. In total, 2494 independent reflections with the range of $2\theta<140°$ were collected by the use of the $2\theta-\omega$ scan mode with a scanning rate of $8°$ (2θ) min^{-1}. A total of 2038 independent reflections with $|F_o|>3\delta(|F_o|)$ was obtained and corrected for Lorentz and polarization factors but not for absorption. The structure was solved by a direct method using MULTAN.[6] The E-map of the phase set with the highest figure of merit showed the skeletons of the molecules. The structure, thus obtained, was refined by the block-diagonal least-squares method with anisotropic temperature factors. After all the hydrogen atoms had been located in

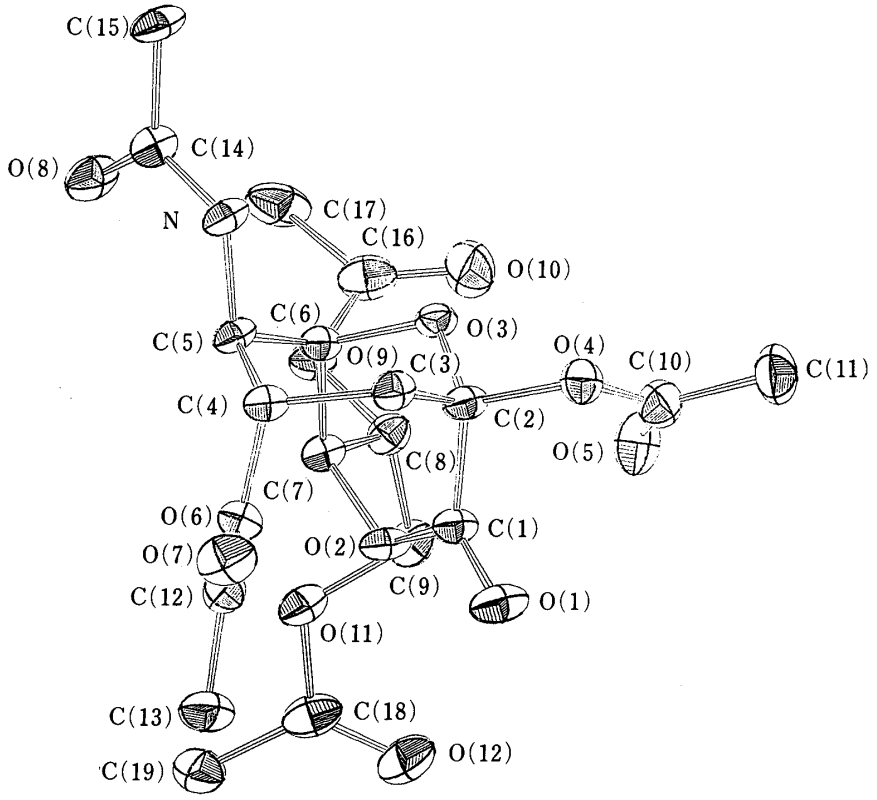


Fig. 2. A Perspective View of the Molecule of **5** and the Atomic Numbering

TABLE II. Atomic Coordinates (10^4) and Their Standard Deviations in Parentheses and Equivalent Isotropic Temperature Factors

Atom	x	y	z	B_{eq}	Atom	x	y	z	B_{eq}
C(1)	4920 (4)	2540 (4)	3906 (7)	3.2	O(3)	4795 (3)	987 (2)	4141 (5)	3.0
C(2)	4810 (5)	1668 (4)	3137 (7)	3.2	O(4)	5547 (3)	1530 (3)	2241 (5)	3.8
C(3)	4012 (4)	1672 (4)	2200 (7)	3.3	O(5)	6437 (3)	1841 (4)	4074 (6)	5.6
C(4)	3195 (4)	1748 (4)	3117 (7)	3.1	O(6)	3141 (3)	2593 (3)	3725 (5)	3.5
C(5)	3229 (4)	1153 (4)	4431 (7)	3.0	O(7)	2554 (4)	3044 (3)	1651 (6)	5.4
C(6)	4110 (4)	1125 (4)	5187 (7)	2.9	O(8)	2400 (4)	−94 (3)	5984 (5)	4.8
C(7)	4365 (4)	1878 (4)	6140 (7)	2.8	O(9)	4736 (3)	1037 (3)	8164 (5)	3.4
C(8)	5124 (4)	1639 (4)	7147 (7)	3.4	O(10)	5986 (4)	289 (4)	8142 (8)	6.9
C(9)	5510 (4)	2388 (5)	7978 (8)	3.8	O(11)	4808 (3)	2840 (3)	8700 (5)	3.8
O(1)	5133 (4)	3156 (3)	3248 (5)	4.6	O(12)	5698 (4)	3946 (4)	8864 (8)	7.0
O(2)	4684 (3)	2600 (2)	5315 (5)	3.4	N(1)	3023 (4)	301 (3)	3910 (6)	3.4

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

C(1)–C(2)	1.558 (8)	C(9)–O(11)	1.460 (8)
C(1)–O(1)	1.120 (8)	C(10)–C(11)	1.492 (12)
C(1)–O(2)	1.354 (8)	C(10)–O(4)	1.344 (9)
C(2)–C(3)	1.505 (10)	C(10)–O(5)	1.204 (10)
C(2)–O(3)	1.421 (7)	C(12)–C(13)	1.515 (11)
C(2)–O(4)	1.423 (8)	C(12)–O(7)	1.199 (10)
C(3)–C(4)	1.523 (9)	C(12)–O(8)	1.347 (9)
C(4)–C(5)	1.536 (9)	C(14)–C(15)	1.521 (9)
C(4)–O(6)	1.450 (7)	C(14)–O(8)	1.216 (8)
C(5)–C(6)	1.528 (9)	C(14)–N(1)	1.338 (9)
C(5)–N(1)	1.464 (8)	C(16)–C(17)	1.507 (12)
C(6)–C(7)	1.530 (9)	C(16)–O(9)	1.390 (9)
C(6)–O(3)	1.449 (7)	C(16)–O(10)	1.194 (10)
C(7)–C(8)	1.543 (9)	C(18)–C(19)	1.492 (12)
C(7)–O(2)	1.457 (7)	C(18)–O(11)	1.349 (8)
C(8)–C(9)	1.530 (10)	C(18)–O(12)	1.218 (10)
C(8)–O(9)	1.464 (8)		

TABLE V. Some Torsion Angles in Degrees

O(3)–C(2)–C(3)–C(4)	59.0 (6)
C(2)–C(3)–C(4)–C(5)	−45.0 (7)
C(3)–C(4)–C(5)–C(6)	42.0 (7)
C(4)–C(5)–C(6)–O(3)	−49.8 (7)
C(5)–C(6)–O(3)–C(2)	61.5 (6)
C(6)–O(3)–C(2)–C(3)	−67.5 (6)
O(3)–C(2)–C(1)–O(2)	−22.9 (8)
C(2)–C(1)–O(2)–C(7)	2.3 (8)
C(1)–O(2)–C(7)–C(6)	−14.5 (8)
O(2)–C(7)–C(6)–O(3)	46.6 (6)
C(7)–C(6)–O(3)–C(2)	−69.0 (6)
C(6)–O(3)–C(2)–C(1)	56.6 (6)
H(4)–C(4)–C(5)–H(5)	−83 (5)
H(5)–C(5)–C(6)–H(6)	79 (5)
O(2)–C(1)–C(2)–C(3)	101.8 (6)
C(5)–C(6)–C(7)–O(2)	−79.6 (7)

TABLE IV. Bond Angle (ϕ/°) and Their Standard Deviations in Parentheses

C(1)–O(2)–C(7)	122.6 (4)	O(7)–C(12)–C(13)	127.2 (7)
C(2)–O(3)–C(6)	109.4 (4)	O(8)–C(14)–N(1)	121.7 (6)
C(2)–O(4)–C(10)	118.0 (6)	O(8)–C(14)–C(15)	123.0 (6)
C(4)–O(6)–C(12)	116.0 (5)	N(1)–C(14)–C(15)	115.3 (6)
C(8)–O(9)–C(16)	115.7 (5)	O(9)–C(16)–O(10)	121.9 (7)
C(9)–O(11)–C(18)	115.6 (5)	O(9)–C(16)–C(17)	109.3 (7)
C(5)–N(1)–C(14)	121.5 (5)	O(10)–C(16)–C(17)	128.7 (8)
O(1)–C(1)–O(2)	120.5 (5)	O(11)–C(18)–O(12)	120.3 (7)
O(1)–C(1)–C(2)	121.2 (6)	O(11)–C(18)–C(19)	112.4 (6)
O(2)–C(1)–C(2)	118.0 (5)	O(12)–C(18)–C(19)	127.3 (7)
O(3)–C(2)–O(4)	106.0 (5)	C(5)–N(1)–H(N1)	116 (3)
O(3)–C(2)–C(1)	111.9 (5)	C(14)–N(1)–H(N1)	118 (3)
O(3)–C(2)–C(3)	111.4 (5)	C(2)–C(3)–H(31)	116 (3)
O(4)–C(2)–C(1)	108.3 (5)	C(2)–C(3)–H(32)	114 (4)
O(4)–C(2)–C(3)	108.7 (5)	C(4)–C(3)–H(31)	105 (3)
C(1)–C(2)–C(3)	110.3 (5)	C(4)–C(3)–H(32)	106 (4)
C(2)–C(3)–C(4)	111.0 (5)	H(31)–C(3)–H(32)	104 (5)
O(6)–C(4)–C(3)	109.6 (5)	O(6)–C(4)–H(4)	117 (3)
O(6)–C(4)–C(5)	105.0 (5)	C(3)–C(4)–H(4)	105 (4)
C(3)–C(4)–C(5)	111.3 (5)	C(5)–C(4)–H(4)	109 (3)
N(1)–C(5)–C(4)	107.1 (5)	N(1)–C(5)–H(5)	111 (4)
N(1)–C(5)–C(6)	108.5 (5)	C(4)–C(5)–H(5)	108 (4)
C(4)–C(5)–C(6)	114.2 (5)	C(6)–C(5)–H(5)	108 (4)
O(3)–C(6)–C(5)	110.4 (5)	O(3)–C(6)–H(6)	107 (4)
O(3)–C(6)–C(7)	108.2 (5)	C(5)–C(6)–H(6)	99 (4)
C(5)–C(6)–C(7)	118.0 (5)	C(7)–C(6)–H(6)	114 (4)
O(2)–C(7)–C(6)	113.2 (5)	O(2)–C(7)–H(7)	112 (3)
O(2)–C(7)–C(8)	104.5 (5)	C(6)–C(7)–H(7)	104 (3)
C(6)–C(7)–C(8)	110.7 (5)	C(8)–C(7)–H(7)	113 (3)
O(9)–C(8)–C(7)	103.6 (5)	O(9)–C(8)–H(8)	110 (3)
O(9)–C(8)–C(9)	109.8 (5)	C(7)–C(8)–H(8)	118 (3)
C(7)–C(8)–C(9)	114.2 (5)	C(9)–C(8)–H(8)	101 (3)
O(11)–C(9)–C(8)	108.5 (5)	O(11)–C(9)–H(91)	115 (3)
O(4)–C(10)–O(5)	122.4 (7)	O(11)–C(9)–H(92)	121 (4)
O(4)–C(10)–C(11)	111.1 (7)	C(8)–C(9)–H(91)	106 (3)
O(5)–C(10)–C(11)	126.6 (7)	C(8)–C(9)–H(92)	117 (3)
O(6)–C(12)–O(7)	122.6 (7)	H(91)–C(9)–H(92)	88 (5)
O(6)–C(12)–C(13)	110.1 (6)		

the difference Fourier map except those attached to five methyl groups (they were assigned to have equal thermal parameters, $B=4$ Å^2), several cycles of least-squares refinement were carried out including these hydrogen atoms. The atomic scattering factors were taken from the International Tables for X-Ray Crystallography.[7] The final R value was 5.9%.

The final atomic parameters are listed in Table II. Figure 2 shows a perspective drawing of **5**. As a result, compound **5** was confirmed to be an acetate of the 1,7-lactone maintaining the anomeric configuration of **1** and the ring system was determined to have a $^5C_2$ conformation. Bond lengths and some torsion angles are shown in Tables III, IV and V. No abnormal bond length was found in the structure.

This evidence indicates that the conformation of the pyranose ring is a chair form and the conformation of the tetrahydropyran ring is a half boat form.

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