Reaction Products from Fischer's Methylation of 3-Deoxy-D-glycero-D-galacto-2-nonulosonic Acid (KDN)

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Several compounds were isolated from the Fischer's methylation reaction mixture of 3-deoxy-D-glycero-D-galacto-2-nonulosonic acid (KDN) with hydrochloric acid or methanesulfonic acid in methanol. The major product was methyl (methyl 3-deoxy- β -D-glycero-D-galacto-2-nonulopyranosid)onate (2), which we have already reported. New furan derivatives and a 4,8-anhydro product were also isolated. The structures of these compounds were elucidated by nuclear magnetic resonance spectroscopy and X-ray crystallographic analysis.

Key words sialic acid; Fischer's glycosylation; furan derivative; 4,8-anhydro product; crystalline KDN; β -elimination

Deaminoneuraminic acid, 3-deoxy-D-glycero-D-galacto-2-nonulosonic acid (KDN, **1**), was isolated from polysialoglycoprotein (PSGP) of rainbow trout eggs.¹⁾ It has been synthesized,^{2,3)} and we have also synthesized various analogues.^{3–8)} These KDN analogues are of particular relevance to the investigation of neuramidase inhibitors,⁹⁾ and they also provide interesting carbohydrate templates for further transformation. KDN methyl ester and its methyl β -glycoside are extremely useful materials for the synthesis of KDN analogues. In a previous paper, we described their synthesis under Fischer's conditions.⁶⁾

In this report, we describe the isolation of minor products from the reaction mixture and determination of their structures by means of mass and proton nuclear magnetic resonance (¹H-NMR) spectroscopy and X-ray crystallographic analysis.

Results and Discussion

Synthesis of carbohydrates requires a large quantity of starting material, because the overall yield is usually low. We developed an improved chemical synthesis of KDN by base-catalyzed condensation of oxalacetic acid with D-mannose,²⁾ obtaining 100 g quantities,³⁾ and we succeeded in crystallizing the β -pyranose form of **1**.

Previously, we examined Fischer's methyl glycosylation of **1** using the strong cation exchange resin, Dowex-50 (H⁺), and we found an anomeric equilibrium between α - and β -furanose and pyranose.⁶⁾ Thus we suspected that the β -pyranoside of methyl (methyl 3-deoxy-D-glycero-D-galacto-2-nonulopyranosid)onate (**2**) would be the only product if **1** were treated with a strong acid, such as hydrochloric acid or methanesulfonic acid, in methanol

1

Fig. 1

8

9

Chart 1

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Chart 2. Possible Mechanism for the Formation of 6 and 7

and heated. We thus found a facile method for the preparation of **2** as new crystals. Furthermore, from the reaction mixture, new furan derivatives and a 4,8-anhydro compound were isolated as minor products.

Treatment of **1** with hydrochloric acid in methanol at 70 °C gave one major and five minor products. The major product, **2**, was crystallized in 28% yield from the concentrated reaction mixture, and the filtrate was separated by column chromatography on silica gel to give three known compounds, the α -isomer of **2** (**3**), and methyl (methyl 3-deoxy- β - and - α -D-glycero-D-galacto-2-nonulofuranosid)onates (**4** and **5**) in 26% total yield, together with two new compounds, methyl 5-(α - and β -D-erythrofuransyl)furan-2-carboxylates (**6**, 0.2% yield and **7**, 0.9% yield). The structures of **6** and **7** were elucidated by $^1\text{H-NMR}$ spectral analysis. The configuration at C-6 of the furan derivatives (**6** and **7**) was deduced from nuclear Overhauser effect (NOE) experiments. That is, on irradiation of H-6, NOEs were observed at H-9 in both the α - and β -anomers (α -anomer **6**, 1.1%; β -anomer **7**, 1.7%). But, on irradiation of H-9, NOE was observed at H-6 only in the β -anomer (**7**, 2.3%), and not in the α -anomer. Furthermore, the structures of **6** and **7** were confirmed by comparison of the $^1\text{H-NMR}$ spectral properties with those of methyl 5-(2',3'-di-O-acetyl- α - and - β -D-erythrofuransyl)pyrrole-2-carboxylates, which are thermal degradation products of sodium *N*-acetylneuraminic acid.¹⁰ The reaction mechanism is considered to involve the formation of a furan ring through acid-catalyzed β -elimination of hydroxy groups and nucleophilic attack of the hydroxy group at C-9 upon the C-6 position, as shown in Chart 2.

In the case of treatment with methanesulfonic acid, instead of hydrochloric acid, in methanol at 70 °C, **1** was converted into a mixture of **2**, **3**, **4**, and **5** in 67% total yield, and a new compound, methyl (methyl 4,8-anhydro-3-deoxy- α -D-glycero-D-galacto-2-nonulofuranosid)onate

Table 1. $^1\text{H-NMR}$ Data for **8** and **9**

Proton	8	9
3-H	2.47 (dd, $J=4.0, 14.5$ Hz)	2.28 (dd, $J=4.0, 14.5$ Hz)
3-H'	2.85 (dd, $J=2.0, 14.5$ Hz)	2.60 (dd, $J=2.0, 14.5$ Hz)
4-H	4.27 (dd, $J=2.0, 4.0$ Hz)	4.22 (dd, $J=2.0, 4.0$ Hz)
5-H	4.69 (dd, $J=2.0, 3.5$ Hz)	4.45 (dd, $J=2.0, 3.5$ Hz)
6-H	4.23 (dd, $J=3.5, 9.5$ Hz)	5.14 (dd, $J=3.5, 9.5$ Hz)
7-H	4.52 (t, $J=9.5$ Hz)	5.37 (t, $J=9.5$ Hz)
8-H	3.77 (dt, $J=3.0, 9.5$ Hz)	3.56 (dt, $J=4.0, 9.5$ Hz)
9-H	4.17 (dd, $J=3.0, 9.5$ Hz)	4.08 (2H, d, $J=4.0$ Hz)
9-H'	4.53 (dd, $J=3.0, 9.5$ Hz)	
2-OMe	3.36 (s)	3.27 (s)
2-COOMe	3.65 (s)	3.82 (s)
OAc	—	2.03 (s), 2.06 (s), 2.10 (s)

Spectra were measured in $\text{C}_5\text{D}_5\text{N}$ for **8** and in CDCl_3 for **9** at 300 MHz. Chemical shifts are given in δ (ppm). Coupling constants are given in parentheses.

(**8**) was also isolated in 3% yield. Acetylation of **8** with acetic anhydride and pyridine afforded methyl (methyl 6,7,9-tri-O-acetyl-4,8-anhydro-3-deoxy- α -D-glycero-D-galacto-2-nonulopyranosid)onate (**9**). The structures of **8** and **9** were elucidated by $^1\text{H-NMR}$ spectral analysis, as shown in Table 1. The coupling constants between H-5 and H-6 ($J=3.5$ Hz), H-6 and H-7 ($J=9.5$ Hz), and H-7 and H-8 ($J=9.5$ Hz) indicate a manno-type proton network for **8**. Further, the coupling between H-4 and H-5 ($J=2.0$ Hz) shows H-4 to be axial. Three singlet signals at δ 2.03, 2.06, and 2.10 of **9** indicate the presence of three newly introduced O-acetyl groups.

To confirm the stereochemistry of **9**, X-ray crystallography was conducted. Figure 2 shows the crystal structure of **9**. From this ORTEP view, the pyranoside takes chair form (4C_4), and the furanoside takes twist form (0T_4), and the anomeric configuration is α .

We assume that **8** is also formed from the open structure of KDN methyl ester in a multistep process, as shown in

Chart 3. The initial step could involve acid-catalyzed β -elimination of the hydroxy group at C-4 and the formation of an α,β -unsaturated keto moiety. Subsequent steps would include nucleophilic attack of the hydroxy group at C-8 on the C-4 position, resulting in a new anhydro ring through intramolecular Michael reaction, and the formation of the furanosyl ring. In this process, none of the original configuration of **8** is changed.

In conclusion, we have developed a facile method for the preparation of crystalline KDN and its β -methyl glycoside, which are required for syntheses of sialic acid analogues. The formation of bicyclic derivatives (**6**, **7**, and **8**) on Fischer methylation is a consequence of the characteristic properties of KDN.

Experimental

Melting points were measured with a Yazawa BY-10 melting point apparatus without correction. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. Thin layer chromatography (TLC) was performed on silica gel 60 F₂₅₄ (Merck) plates, and spots were detected under ultraviolet (UV) light and by spraying with 5% sulfuric

acid. Fast atom bombardment mass spectra (FAB-MS), and infrared (IR) spectra were measured with JEOL JMS-DX300 and JASCO A-102 instruments, respectively. The ¹H-NMR spectra were measured with Varian VXR-300 spectrometer. Tetramethylsilane (TMS) in CDCl₃ and C₃D₅N or sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) in D₂O were used as internal references. Column chromatography was conducted on Silica gel 60 (70–230 mesh, Merck).

Purification of KDN A solution of **1** (10.0 g, 37.3 mmol) in water (100 ml) was passed over the anion exchange resin Dowex-1 (HCOO⁻, 350 ml), and **1** was eluted with 0–1 N formic acid. The eluate was concentrated under reduced pressure. The residue was left at room temperature for 7 d, to afford colorless crystals (4.65 g, 47%). mp 144–147 °C. $[\alpha]_D^{30} -49.7^\circ$ ($c=1$, H₂O). Anal. Calcd for C₉H₁₆O₉: C, 40.30; H, 6.01. Found: C, 40.39; H, 5.89. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3440, 2960, 1730. ¹H-NMR (300 MHz, D₂O) δ : 1.75 (1H, dd, $J=11.5$, 12.5 Hz, 3-H_{ax}), 2.18 (1H, dd, $J=5.0$, 12.5 Hz, 3-H_{eq}), 3.51 (1H, t, $J=9.5$ Hz, 5-H), 3.58 (1H, dd, $J=6.0$, 11.5 Hz, 9-H), 3.67 (1H, ddd, $J=3.0$, 6.0, 9.0 Hz, 8-H), 3.79 (1H, dd, $J=1.5$, 9.0 Hz, 7-H), 3.79 (1H, dd, $J=3.0$, 11.5 Hz, 9-H'), 3.91 (1H, dd, $J=1.5$, 9.5 Hz, 6-H), 3.92 (1H, ddd, $J=5.0$, 9.5, 11.5 Hz, 4-H).

Esterification and Glycosylation of 1 by Fischer's Method Using Hydrochloric Acid and Methanol Acetyl chloride (22.08 g, 0.281 mol) was added slowly to methanol (500 ml), then KDN (**1**, 10.0 g, 37.3 mmol) was added to the solution. The mixture was heated at 70 °C for 6 h, then evaporated, and the residual syrup was left at room temperature to afford methyl (methyl 3-deoxy- β -D-glycero-D-galacto-2-nonulopyranosid)onate (**2**, 3.04 g, 28%) as colorless crystals. These were filtered off, and the filtrate was evaporated to dryness. The residual syrup was purified on a column of silica gel with chloroform–methanol (10:1) to give a mixture of methyl 5-(α - and β -D-erythrofuranosyl)furan-2-carboxylates (**6** and **7**, 216.9 mg, 3%), a mixture of methyl (methyl 3-deoxy- β - and α -D-glycero-D-galacto-2-nonulopyranosid)onate (**2** and **3**) and a mixture of methyl (methyl 3-deoxy- β - and α -D-glycero-D-galacto-2-nonulofuranosid)onate (**4** and **5**) (2.9 g, 26%). The mixture of **6** and **7** was separated by silica gel TLC with 20:1 (v/v) chloroform–methanol (repeated 3 times) to give **6** (12.9 mg, 0.2%) and **7** (78.3 mg, 0.9%).

6: $[\alpha]_D^{30} -7.6^\circ$ ($c=1$, MeOH). HRMS m/z Calcd for C₁₀H₁₂NaO₆: 251.0532 (M⁺ + Na). Found: 251.0533 (M⁺ + Na). IR $\nu_{\max}^{\text{film}}$ cm⁻¹: 3420, 1740, 1610. ¹H-NMR (300 Mz, C₃D₅N) δ : 3.74 (3H, s, -COOCH₃), 4.22 (1H, dd, $J=6.5$, 8.5 Hz, 9-H), 4.30 (1H, dd, $J=6.5$, 8.5 Hz, 9-H'), 4.67 (1H, t, $J=4.5$ Hz, 7-H), 4.72 (1H, dt, $J=4.5$, 6.5 Hz, 8-H), 5.27 (1H, d, $J=4.5$ Hz, 6-H), 6.90 (1H, d, $J=3.5$ Hz, 4-H), 7.30 (1H, d, $J=3.5$ Hz, 3-H).

7: $[\alpha]_D^{30} -59.4^\circ$ ($c=1$, MeOH). HRMS m/z Calcd for C₁₀H₁₂NaO₆: 251.0532 (M⁺ + Na). Found: 251.0544 (M⁺ + Na). IR $\nu_{\max}^{\text{film}}$ cm⁻¹: 3450, 2980, 1740, 1660, 1540. ¹H-NMR (300 Mz, C₃D₅N) δ : 3.76 (3H, s, -COOCH₃), 4.26 (1H, dd, $J=2.5$, 9.0 Hz, 9-H), 4.42 (1H, dd, $J=5.0$, 9.0 Hz, 9-H'), 4.65 (1H, dt, $J=2.5$, 9.0 Hz, 8-H), 4.80 (1H, t, $J=5.0$, 7.0 Hz, 7-H), 5.34 (1H, d, $J=7.0$ Hz, 6-H), 6.66 (1H, d, $J=3.5$ Hz, 4-H), 7.24 (1H, d, $J=3.5$ Hz, 3-H).

Esterification and Glycosylation of 1 by Fischer's Method Using Methanesulfonic Acid and Methanol A solution of **1** (5.1 g, 19.0 mmol) and methanesulfonic acid (1 ml) in methanol was stirred for 8 h at 70 °C, then neutralized with dry Dowex-1 (OH⁻) and filtered. The filtrate was evaporated and the residual syrup was chromatographed on a column of silica gel with chloroform–methanol (4:1) to give methyl (methyl

Fig. 2. Crystal Structure of **9**

Chart 3. Possible Mechanism for the Formation of **8**

4,8-anhydro-3-deoxy- α -D-glycero-D-galacto-2-nonulofuranosid)onate (**8**, 140 mg, 3%) and a mixture of **2**, **3**, **4**, and **5** (3.8 g, 67%).

8: $[\alpha]_D^{23} - 53.6^\circ$ ($c=1$, MeOH). HRMS m/z Calcd for $C_{11}H_{19}O_8$: 279.1080 ($M^+ + H$). Found: 279.1104 ($M^+ + H$). IR $\nu_{\max}^{CHCl_3} \text{ cm}^{-1}$: 3450, 1745. $^1\text{H-NMR}$ data are given in Table 1.

Acetylation of 8 Acetic anhydride (165 mg, 1.62 mmol) was added to a solution of **8** (50.0 mg, 0.180 mmol) in pyridine (128 mg, 1.62 mmol) at room temperature. The mixture was stirred for 16 h at room temperature, then concentrated, and the residue was purified on a column of silica gel with *n*-hexane-ethyl acetate (1:1) to give methyl (methyl 6,7,9-tri-*O*-acetyl-4,8-anhydro-3-deoxy- α -D-glycero-D-galacto-2-nonulofuranosid)onate (**9**, 30 mg, 41%) as colorless prisms. mp 133–135°C, $[\alpha]_D^{23} - 73.2^\circ$ ($c=1$, MeOH). Anal. Calcd for $C_{17}H_{24}O_{11}$: C, 50.50; H, 5.94. Found: C, 50.49; H, 6.02. IR $\nu_{\max}^{CCl_4} \text{ cm}^{-1}$: 1750. $^1\text{H-NMR}$ data are given in Table 1.

Crystal Data for 9 A crystal with the dimensions of $0.3 \times 0.3 \times 0.3 \text{ mm}^3$ was used for the structure determination. The cell dimensions and diffraction intensities were measured on a Rigaku four-circle diffractometer (AFC-5R), using graphite-monochromated $\text{CuK}\alpha$ radiation.

Crystal Data: $C_{17}H_{24}O_{11}$, orthorhombic, space group $P2_12_12_1$, $a=9.625(4)$, $b=25.930(9)$, $c=7.802(5) \text{ \AA}$, $V=1947(1) \text{ \AA}^3$, $Z=4$, $D_{\text{calcd}}=1.379 \text{ g/cm}^3$. In total, 2053 independent reflections in the range of $2\theta=139.6^\circ$ were collected by use of the $2\theta-\omega$ scan mode with a scanning rate of $16^\circ \text{ min}^{-1}$ (ω). In total, 1629 independent reflections with $I>3.00\sigma(I)$ were obtained and corrected for Lorentz and polarization factors, but not for absorption. The structure was solved by direct methods using the program MITHRIL.¹¹⁾ The positions of all hydrogen atoms were located in the difference Fourier map. Atomic scattering factors were taken from the International Table for X-Ray Crystallography.¹²⁾ All calculations were performed using the TEXSAN¹³⁾ crystallographic software package of Molecular Structure Corporation. The final R value was 5.3%.

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