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Note

Synthesis of γ-Lactone-Type Sialic Acid, an Isomer of 2,3-Dehydrosialic Acid

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An efficient synthesis of γ-lactone-type sialic acid, which is an isomer of 2,3-dehydrosialic acid, was achieved from the corresponding sialic ester. The sialic ester was reconstructed from the γ-lactone in a 95% yield. The γ-lactone structure was determined by methylating to its corresponding methyl ether.

Key words: γ-lactone-type sialic acid; 2,3-dehydrosialic acid; isomerization

Sialic acid (*N*-acetylneuraminic acid, **1**) is widely distributed in animal tissues as glycoproteins and gangliosides and plays an important role in human influenza infections. The structure of sialic acid is a unique C_9-carbohydrate that can be biologically synthesized from *N*-acetylmannosamine and pyruvic acid, and that exists as a mixture of two anomers.[1] However, sialic acid (**1**) exists primarily as the β-form in a solution. 2,3-Dehydrosialic acid[2,3] is one of the sialic acid derivatives and has interesting bioactivities. The reactivity of sialic acid (**1**) was evaluated in this study, since **1** and its derivatives are medicinally interesting compounds.

We first examined the reactivity of sialic acid (**1**) and its sialic ester (**2**). Sialic ester (**2**) was easily prepared from sialic acid in methanol containing either HCl[4] or an acidic resin.[5] Compound **1** or **2** was treated with many reagents under various reaction conditions, and a new clean spot was found on a silica gel TLC plate upon treating **2** with 0.5 M sodium methoxide in methanol for 30 min. However, the major product decomposed during silica gel p-TLC or column chromatography separation and could not be isolated from the reaction mixture. Changing to crystallization allowed us to isolate the unstable crystalline product upon neutralization with CK08P acidic resin. The UV absorption of this crystal was observed at 229 nm (λ_{max}, $\varepsilon = 6{,}800$), while the proton signal for the methyl ester disappeared and a vinyl proton signal appeared at 6.53 ppm (doublet, $J = 2.1$ Hz) in the ^{1}H-NMR spectrum, suggesting the presence of a conjugated lactone.

Ogura *et al.*[6,7] reported in 1987 the synthesis of compound **4a**. We therefore prepared the methyl ether by methylating that crystalline compound to determine the structure. Treating the crystalline compound with diazomethane in methanol afforded corresponding methyl ether **4a** in a 96% yield. The ^{1}H-NMR spectrum of purified methyl ether **4a** was identical to the data reported by Ogura *et al.* The result proved to be γ-lactone **3**, and the yield from **2** was 62%.

Two mechanisms can be proposed for the synthesis of γ-lactone **3** from **2** *via* bicyclo-intermediate **I** or ring-opened intermediate **II** in Scheme 1. The instability of γ-lactone **3** suggested that the original pyranose-type backbone found in sialic acid may be reconstructed from linear conjugated γ-lactone **3**.

We attempted to recover sialic acid by treating γ-lactone **3** with an aqueous alkali solution like NaOH, KOH or $NaHCO_3$, but the yield was low. The ring-construction reaction was performed in various methanolic alkali solutions containing NaOH, $NaHCO_3$ or NEt_3, achieving a spot identical with that of methyl ester **2**. The product was purified by silica gel column chromatography to produce pure sialic ester **2** in a 95% yield, its structure being confirmed by ^{1}H-NMR.

Methyl ether **4a** was isomerised into an equilibrium mixture (**4a**/**4b** = 3/1) in 1% triethylamine-methanol at 110 °C for 4 h in a sealed tube (Scheme 1). Treating lactone **4a** with *n*-butylamine enabled a ring-opening reaction to proceed, affording linear conjugated amide **5** in an 81% yield (Scheme 1). 2,3-Dehydrosialic acid analogues **3**, **4a**, **4b**, and **5** may be more activated structures than the original sialic acid itself, because their conjugated γ-lactone or amide function is a potent Michael accepter. Further chemical studies on the application of these dehydrosialic acid derivatives are currently in progress.

Experimental

All reactions were carried out in an N_2 atmosphere. Silica gel column chromatography was performed with silica gel M-300H (Nagara Science Co.), and optical rotation values were recorded by a JASCO P-2300 digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were measured by a JEOL ECA 600 spectrometer, with tetramethylsilane used as an internal standard in CD_3OD or *t*-BuOH in D_2O. Mass spectra were recorded with a JMS-700 (ESI) spectrometer, and UV spectra were recorded with a Hitachi 4000 U spectrophotometer. Melting point (mp) data were measured with Yanaco MP-J3 micro-melting point apparatus.

*γ-Lactone-type sialic acid (**3**).* Five ml of 1 M CH_3ONa in CH_3OH was added to a solution of **2** (1.0 g, 3.1 mmol) in anhydrous CH_3OH (5.0 ml), and the mixture was stirred at 25 °C for 30 min. The base was trapped with an CK08P acidic resin (500 mg) column and washed 3 times with anhydrous CH_3OH (30 ml). After evaporating the solvent, the crude product was recrystalized from CH_3OH/CH_2Cl_2 (1/4) to give 0.56 g (62%) of **3** as colorless prisms. ^{1}H-NMR (600 MHz, D_2O) δ: 6.53 (1H, d, $J = 2.1$ Hz, H-3), 5.81 (1H, t, $J = 2.1$ Hz, H-4), 4.60 (1H, dd, $J = 10.3$, 2.1 Hz, H-5), 4.11 (1H, dd, $J = 12.4$, 2.8 Hz, H-9), 4.03 (1H, m, H-8), 3.89 (1H, dd, $J = 11.7$, 6.2 Hz, H-9), 3.77 (1H, d, $J = 8.3$ Hz, H-7), 2.20 (3H, s, N-Ac); ^{13}C-NMR (150 MHz, D_2O) δ: 170.98 (C-1), 170.34 ($COCH_3$), 143.63 (C-2), 118.75 (C-3), 78.31 (C-4), 72.27 (C-8), 70.69 (C-7), 68.92 (C-6), 64.77 (C-9), 51.76 (C-5), 23.43 ($COCH_3$). HR-ESI-MS (positive): calcd. for $C_{11}H_{17}NNaO_8$

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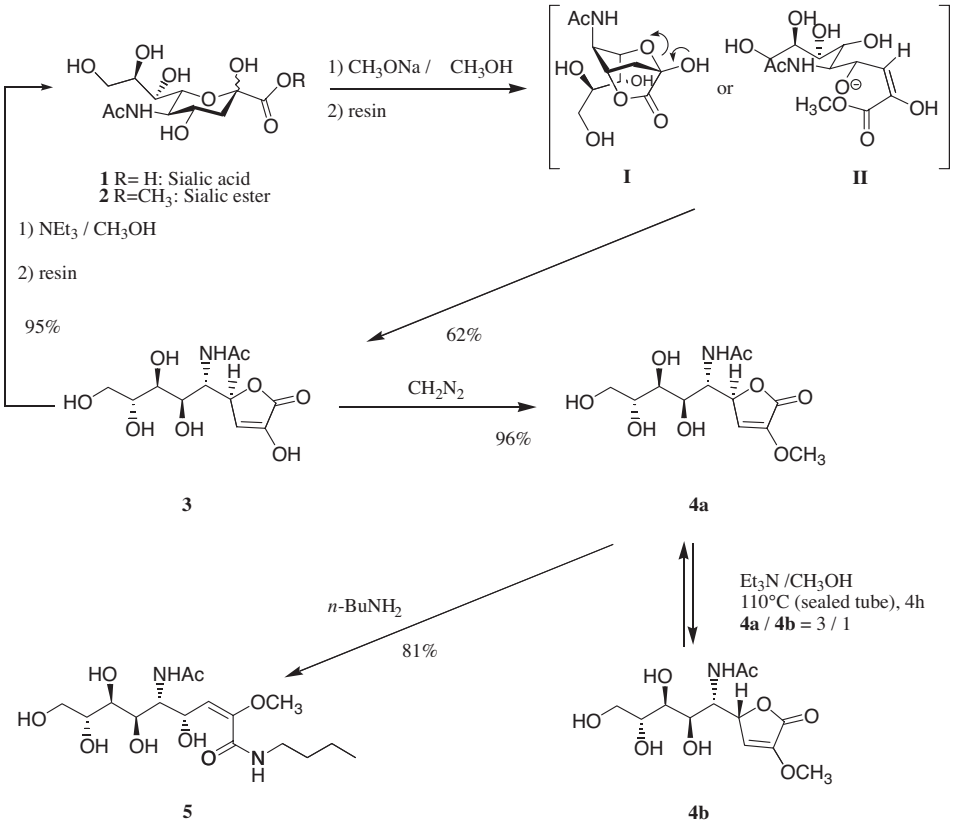

Scheme 1. Synthesis of γ-Lactone-Type Sialic Acids (**3** and **4ab**) and Derivative **5**.

(M + Na), 314.0852; found, *m/z* 314.0859. Mp 192 °C (decomp.); UV (CH_3OH) λ_{max} 229 nm (ε 6,800); $[\alpha]_D^{20}$ −137° (c 1.0, CH_3OH).

N-*Acetylneuraminic acid methyl ester (**2**).* Triethylamine (25 µl) was added dropwise to **3** (100 mg, 0.34 mmol) in CH_3OH (5 ml), and the mixture stirred for 30 min at 25 °C. After removing the solvent, the residue was purified by silica gel column chromatography with 5% CH_3OH/CH_2Cl_2 to afford pure sialic ester **2** (105 mg, 95%), whose structure was confirmed by ^{1}H-NMR. The respective yields of **2** using NaOH or $NaHCO_3$ were 92% and 93%.

*2-O-Methyl ether (**4a**).* Five ml of an ethereal solution of diazomethane was added to **3** (50 mg, 0.17 mmol) in CH_3OH (5 ml). After stirring the mixture for 30 min at 25 °C, 1 drop of acetic acid was added to terminate the reaction. After evaporation, the residue was purified by silica gel open column chromatography with 5% CH_3OH in CH_2Cl_2 to give 2-*O*-methyl derivative **4a** (50.3 mg, 96%) as a colorless syrup. The ^{1}H-NMR spectrum of **4a** was identical with that of **4a** reported by Ogura *et al.*[6)]

*Isomerization of 2-O-methyl ether (**4a**).* Triethylamine (10 µl) was added to a solution of **4a** (50 mg, 0.16 mmol) in CH_3OH (1 ml), and the mixture was heated at 110 °C in a sealed tube. The reaction was monitored by ^{1}H-NMR for 4 h until the mixture had been converted to a 3/1 equilibrium mixture (**4a**/**4b**). Neither **4a** nor **4b** could be isolated, so the mixture was acetylated overnight with Ac_2O/pyridine at 20 °C. The peracetates were isolated to the natural R-isomer (**4a** peracetate) and unnatural S-isomer (**4b** peracetate).

4a peracetate. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 5.96 (1H, d, J = 2.1 Hz, H-3), 5.59 (1H, d, J = 10.3 Hz, NH), 5.50 (1H, dd, J = 9.6, 2.1 Hz, H-6), 5.41 (1H, dd, J = 8.9, 2.1 Hz, H-7), 5.08 (1H, m, H-8), 4.91 (1H, t, J = 2.1 Hz, H-4), 4.53 (1H, t, J = 10.3 Hz, H-5), 4.24 (1H, dd, J = 12.4, 2.8 Hz, H-9), 4.01 (1H, dd, 12.4, 5.5 Hz, H-9), 2.16, 2.11, 2.08, 2.06 [4 × (3H, s), 4 × -OAc], 1.88 (3H, s, N-Ac).

4b peracetate. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 6.10 (1H, d, J = 2.1 Hz, H-3), 5.81 (1H, brd, J = 9.6 Hz, NH), 5.41 (1H, dd, J = 8.3, 2.8 Hz, H-6), 5.39 (1H, dd, J = 7.6, 2.8 Hz, H-7), 5.06 (1H, m, H-8), 4.97 (1H, dd, J = 2.1, 4.1 Hz, H-4), 4.55 (1H, dt, J = 9.6, 4.1 Hz, H-5), 4.25 (1H, dd, J = 12.4, 3.4 Hz, H-9), 4.02 (1H, dd, J = 12.4, 5.5 Hz, H-9), 2.14, 2.11, 2.06, 2.05 [4 × (3H, s), 4 × -OAc], 1.96 (3H, s, N-Ac).

*Open-chain conjugated amide of sialic acid (**5**).* *n*-Butylamine (18 µl) was added to a solution of **4a** (50 mg, 0.16 mmol) in CH_3OH (0.5 ml), and the mixture stirred for 2 h at 25 °C while being monitored by HPLC. After evaporating the solvent, the product was purified by HPLC (35% CH_3OH, 1% AcOH/H_2O) to give amide **5** (50.3 mg, 81%) as a colorless syrup. ^{1}H-NMR (CD_3OD) δ: 5.40 (1H, dd, J = 7.6, 2.1 Hz, H-4), 5.14 (1H, d, J = 7.6 Hz, H-3), 4.02 (1H, dd, J = 9.6, 2.1 Hz, H-5), 3.96 (1H, d, J = 10.3 Hz, H-6), 3.80 (1H, dd, J = 11.2, 3.4 Hz, H-9a), 3.72 (1H, m, H-8), 3.62 (1H, dd, J = 11.2, 6.2 Hz, H-9b), 3.59 (3H, s, OCH_3), 3.43 (1H, d, J = 8.9 Hz, H-7), 3.24 (1H, dt, J = 6.9, 2.1 Hz, N-CH_2-C_3H_7), 2.02 (3H, s, H-Ac), 1.52 (2H, m, N-CH_2-CH_2-C_2H_5), 1.36 (2H, m, N-C_2H_4-CH_2-CH_3), 0.94 (3H, t, J = 7.6 Hz, N-C_3H_6-CH_3). HR-ESI-MS (positive): calcd. for $C_{16}H_{30}N_2NaO_8$ (M + Na), 401.1900; found, *m/z* 401.1896. $[\alpha]_D^{20}$ −30° (c 1.0, CH_3OH).

Acknowledgments

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