Synthesis of Pyridinium Dinitrobenzyl Sulfates and Potassium (Dinitrobenzyl β-D-Glucopyranosid)Uronates

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Sulfates and glucuronides of 2,4-dinitrobenzyl alcohol 1a and 2,6-dinitrobenzyl alcohol 1b, which are major or putative metabolites of 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoluene (2,6-DNT), were synthesized from 1a and 1b by reaction with pyridinium sulfonate and methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl)uronate bromide 3, respectively, as their pyridinium salts (2a, 2b) and potassium salts (6a, 6b). These conjugates are important for the study of the carcinogenicity of 2,4-DNT and 2,6-DNT.

Key words dinitrobenzyl sulfate; dinitrobenzyl glucuronide; dinitrotoluene; carcinogenicity

The glucuronides of dinitrobenzyl alcohols (**1a**, **1b**), which are major urinary and biliary metabolites in rats dosed with 2,4-dinitrotoluene (2,4-DNT)[1] and 2,6-dinitrotoluene (2,6-DNT),[2] have been postulated to be precursors of mutagenic metabolites of 2,4-DNT[3] and 2,6-DNT.[4] In addition, the glucuronide of **1b** has been shown to be an intermediary metabolite responsible for the carcinogenicity of 2,6-DNT.[5] However, these glucuronides have not been synthesized chemically. Moreover, metabolites having a hydroxyl group are known to undergo sulfation in the metabolic process.[6]

It seems likely that the secondary metabolism of these conjugates is essential for investigation of the toxic action of 2,4-DNT and 2,6-DNT. In this note, we report the synthesis of pyridinium dinitrobenzyl sulfates (**2a**, **2b**) and potassium (dinitrobenzyl β-D-glucopyranosid)uronates (**6a**, **6b**).

Synthesis of Pyridinium Dinitrobenzyl Sulfates (2a, 2b) 2,4-Dinitrobenzyl sulfate and 2,6-dinitrobenzyl sulfate, as free sulfates, were synthesized from **1a** and **1b**, respectively, by sulfonation with chlorosulfonic acid in methylene chloride, but they were highly hygroscopic. Thus, the sulfates of **1a** and **1b** were synthesized as their pyridinium salts (**2a**, **2b**), as shown in Chart 1. Pyridinium 2,4-dinitrobenzyl sulfate **2a** and its isomer **2b** were synthesized from **1a** and **1b** by sulfonation with pyridinium

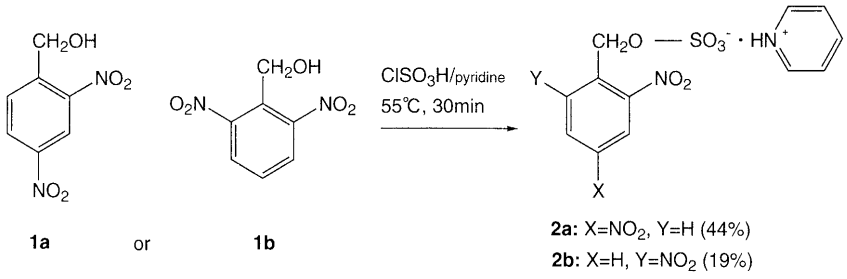

Chart 1

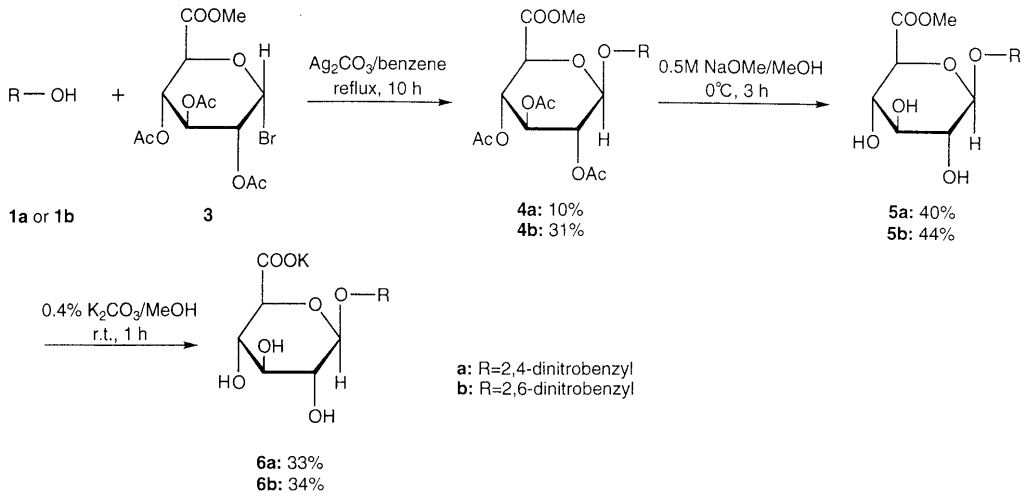

Chart 2

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sulfonate in pyridine, in yields of 44 and 19%, respectively. The signals of the pyridinium protons appeared as doublets and triplets at δ 8.00—8.88 in the ^{1}H-NMR spectra. Strong peaks due to the sulfates appeared at 1232 and 1238 cm^{-1}, respectively, in the IR spectra.

Synthesis of Potassium (Dinitrobenzyl β-D-Glucopyranosid)uronates (6a, 6b) Potassium (2,4-dinitrobenzyl β-D-glucopyranosid)uronate **6a** and its isomer **6b** were synthesized *via* the route shown in Chart 2. The reaction of **1a** and **1b** with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl)uronate bromide **3** according to Bollenback's procedure[7] in the presence of silver carbonate gave **4a** and **4b**, in yields of 10 and 31%, respectively. Kanaoka *et al.*[8] have shown that 18β-glycyrrhetyl glucuronide is obtained directly by hydrolysis of the condensation product of glycyrretic acid and **3** with 5% KOH in MeOH. However, since the treatment of **4a** and **4b** with the methanolic 5% KOH gave **1a** and **1b**, the deprotection of **4a** and **4b** was performed stepwise. The solvolysis of **4a** and **4b** with sodium methoxide afforded **5a** and **5b** in yields of 40 and 44%, respectively. Subsequently, the hydrolysis of **5a** and **5b** with potassium carbonate gave **6a** and **6b** in 33 and 34% yields, respectively. The signals of the anomeric protons of **6a** and **6b** appeared as doublets at δ 4.38 (J=7.5 Hz) and δ 4.29 (J=7.7 Hz), respectively. β-Configuration of the glucuronide linkages of **6a** and **6b** was supported by the optical rotation and the fact that they were hydrolyzable with β-glucuronidase.

In conclusion, the chemical synthesis of these conjugates may be useful for studies on the active species related to the carcinogenicity of 2,4-DNT and 2,6-DNT.

Experimental

All melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were recorded with a JASCO FT/IR-7000 spectrometer, and UV spectra with a Hitachi 150-20 spectrometer. Optical rotations were recorded with a JASCO DIP-4 digital polarimeter. ^{1}H-NMR spectra were recorded with a Varian Unity-5000 spectrometer, with tetramethylsilane as an internal standard. MS were recorded with a JEOL JMS-D300 spectrometer. Wakogel C-200 (silica gel) and Merck Kieselgel 60F$_{254}$ (silica gel, aluminum sheet) were used for column chromatography and thin layer chromatography (TLC), respectively. Enzymic hydrolysis was carried out as follows: glucuronides (**6a** and **6b**, each 1.3 μmol) were incubated at 37 °C for 10 h in 0.2 M sodium acetate buffer (pH 5) with β-glucuronidase (1000 U); dinitrobenzyl alcohols (**1a**, **1b**) liberated were detected by TLC ($CHCl_3$: MeOH = 9 : 1) and HPLC (ODS-80TM column, MeCN : H_2O = 1 : 1). Compounds **1a**,[9] **1b**[10] and **3**[7] were prepared by methods described previously.

Pyridinium 2,4-Dinitrobenzyl Sulfate (2a) A solution of 2,4-dinitrobenzyl alcohol **1a** (3.0 g, 152 mmol) in dry pyridine (3 ml) was added to pyridinium sulfonate, which was prepared from dry pyridine (18 ml) and $ClSO_3H$ (5.3 g, 45.5 mmol),[11] and the mixture was kept at 55 °C for 30 min, then evaporated *in vacuo*. The residue was recrystallized from MeOH to give pure **2a** (pale brownish plates, mp 134—135 °C, 2.4 g, 44%). UV λ_{max} (EtOH) nm (ε): 245 (18900). IR (KBr) cm^{-1}: 1531 (NO_2), 1232 (sulfate). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 5.27 (s, 2H, methylene), 8.00 (t, 2H, J=6.7 Hz, pyridinium 3,5-H), 8.07 (d, 1H, J=9.2 Hz, aromatic 6-H), 8.51 (t, 1H, J=7.9 Hz, pyridinium 4-H), 8.61 (dd, 1H, J=2.4, 8.5 Hz, aromatic 5-H), 8.78 (d, 1H, J=2.4 Hz, aromatic 3-H), 8.88 (d, 2H, J=4.9 Hz, pyridinium 2,6-H). MS (FAB) *m/z*: 277 ($M-C_5H_6N^-$). *Anal.* Calcd for $C_{12}H_{11}N_3O_8S$: C, 40.34; H, 3.10; N, 11.76. Found: C, 40.39; H, 3.15; N, 11.85.

Pyridinium 2,6-Dinitrobenzyl Sulfate (2b) 2,6-Dinitrobenzyl alcohol **1b** (2.0 g, 10.0 mmol) was treated with pyridinium sulfonate in the same manner as described for the synthesis of **2a**. The residue obtained was recrystallized from MeOH to give pure **2b** (pale yellowish powder, mp 136—137 °C, 0.7 g, 19%). UV λ_{max} (EtOH) nm (ε): 231 (13400). IR (KBr) cm^{-1}: 1537 (NO_2), 1238 (sulfate). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 5.14 (s, 2H, methylene), 7.78 (t, 2H, J=8.1 Hz, aromatic 4-H), 8.01 (t, 2H, J=7.1 Hz, pyridinium 3,5-H), 8.20 (d, 2H, J=8.3 Hz, aromatic 3,5-H), 8.53 (t, 1H, J=7.8 Hz, pyridinium 4-H), 8.90 (d, 2H, J=4.9 Hz, pyridinium 2,6-H). MS (FAB) *m/z*: 277 ($M-C_5H_6N^-$). *Anal.* Calcd for $C_{12}H_{11}N_3O_8S$: C, 40.34; H, 3.10; N, 11.76. Found: C, 40.21; H, 3.12; N, 11.55.

Methyl (2,4-Dinitrobenzyl 2,3,4-tri-*O*-acetyl-β-D-Glucopyranosid)uronate (4a) Compound **1a** (5.0 g, 2.53 mmol) was dissolved in dry benzene (150 ml). To the boiling solution, a solution of **3** (15.0 g, 37.8 mmol) in dry benzene (150 ml) and freshly prepared Ag_2CO_3 (1.0 g) were added little by little over 10 h. Benzene was distilled off gradually, and stirring was continued. The mixture was filtered, and the filtrate was concentrated to a syrup. The syrup was purified by column chromatography ($CHCl_3$: hexane 1 : 4) to give **4a** (colorless needles, mp 159—160 °C, 1.3 g, 10%). UV λ_{max} (EtOH) nm (ε): 206 (10200), 242 (14700). IR (KBr) cm^{-1}: 1750 (ester), 1543 (NO_2). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 1.98, 2.00, 2.05 (s, 3H each, CH_3CO), 3.64 (s, 3H, OCH_3), 4.50 (d, 1H, J=9.8 Hz, glucuronate 5-H), 4.95 (dd, 1H, J=7.9, 9.6 Hz, glucuronate 2-H), 5.00 (dd, 1H, J=9.6, 9.8 Hz, glucuronate 4-H), 5.06 (d, 1H, J=7.9, Hz, glucuronate 1-H), 5.19 (abq, 2H, J=15.6 Hz, methylene), 5.38 (t, 1H, J=9.6 Hz, glucuronate 3-H), 7.90 (d, 1H, J=8.8 Hz, aromatic 6-H), 8.66 (dd, 1H, J=2.4, 8.5 Hz, aromatic 5-H), 8.79 (d, 1H, J=2.4 Hz, aromatic 3-H). MS (FAB) *m/z*: 514 (M^-). *Anal.* Calcd for $C_{20}H_{22}N_2O_{14}$: C, 46.70; H, 4.31; N, 5.45. Found: C, 46.83; H, 4.30; N, 5.43.

Methyl (2,6-Dinitrobenzyl 2,3,4-tri-*O*-acetyl-β-D-Glucopyranosid)uronate (4b) 2,6-Dinitrobenzyl alcohol **1b** (5.0 g, 25.3 mmol) was reacted with **3** (15.0 g, 37.8 mmol) in the same manner as the synthesis of **4a**. The resulting syrup was purified by column chromatography ($CHCl_3$: hexane = 1 : 3) to give **4b** (colorless needles, mp 162—164 °C, 4.0 g, 31%). UV λ_{max} (EtOH) nm (ε): 205 (13600), 230 (11300). IR (KBr) cm^{-1}: 1750 (ester), 1537 (NO_2). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 1.94, 1.97, 1.99 (s, 3H each, CH_3CO), 3.64 (s, 3H, OCH_3), 4.43 (d, 1H, J=9.9 Hz, glucuronate 5-H), 4.72 (dd, 1H, J=8.2, 9.3 Hz, glucuronate 2-H), 4.88—4.95 (m, 2H, glucuronate 1,4-H), 5.03 (abq, 2H, J=13.2 Hz, methylene), 5.31 (t, 1H, J=9.3 Hz, glucuronate 3-H), 7.87 (t, 1H, J=8.2 Hz, aromatic 4-H), 8.26 (d, 2H, J=8.2 Hz, aromatic 3,5-H). MS (FAB) *m/z*: 514 (M^-). *Anal.* Calcd for $C_{20}H_{22}N_2O_{14}$: C, 46.70; H, 4.31; N, 5.45. Found: C, 46.47; H, 4.40; N, 5.53.

Methyl (2,4-Dinitrobenzyl β-D-Glucopyranosid)uronate (5a) A solution of **4a** (1.0 g, 1.95 mmol) in dry MeOH (200 ml) was treated with 0.5 M NaOMe (4 ml) and the solution was stirred for 3 h in an ice bath. The mixture was neutralized with Dowex 50W × 8 (H^+ form), filtered and concentrated. The residue was recrystallized from EtOAc to give pure **5a** (colorless needles, mp 183—185 °C, 0.3 g, 40%). UV λ_{max} (EtOH) nm (ε): 206 (8800), 243 (13500). IR (KBr) cm^{-1}: 3400 (OH), 1734 (CO), 1535 (NO_2). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 3.16—3.25 (m, 3H, glucuronate 2,3,4-H), 3.67 (s, 3H, OCH_3), 3.82 (d, 1H, J=9.8 Hz, glucuronate 5-H), 4.49 (d, 1H, J=7.5 Hz, glucuronate 1-H), 5.17 (abq, 2H, J=17.1 Hz, methylene), 5.25 (d, 1H, J=8.8 Hz, aromatic 1-H), 8.29 (d, 1H, J=8.8 Hz, aromatic 6-H), 8.58 (dd, 1H, J=2.4 Hz, aromatic 5-H), 8.80 (d, 1H, J=2.4 Hz, aromatic 3-H). MS (FAB) *m/z*: 388 (M^-). *Anal.* Calcd for $C_{14}H_{16}N_2O_{11}$: C, 43.31; H, 4.15; N, 7.21. Found: C, 43.51; H, 4.21; N, 7.07.

Methyl (2,6-Dinitrobenzyl β-D-Glucopyranosid)uronate (5b) Compound **4b** (1.0 g, 1.95 mmol) was treated with 0.5 M NaOMe (200 ml) in the same manner as described for the synthesis of **5a**. The resulting oily residue was purified by column chromatography (EtOAc : $CHCl_3$ = 10 : 3) to give **5b** (pale yellowish foam, 0.33 g, 44%). UV λ_{max} (EtOH) nm (ε): 206 (12700), 229 (10100). IR (KBr) cm^{-1}: 3408 (OH), 1746 (CO), 1535 (NO_2). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 2.86—3.25 (m, 3H, glucuronate 2,3,4-H), 3.62 (s, 3H, OCH_3), 3.65 (d, 1H, J=9.6 Hz, glucuronate 5-H), 4.29 (d, 1H, J=7.7 Hz, glucuronate 1-H), 5.00 (abq, 2H, J=13.5 Hz, methylene), 5.07 (d, 1H, J=5.3 Hz, OH), 5.12 (d, 1H, J=5.6 Hz, OH), 5.30 (d, 1H, J=6.0 Hz, OH), 7.81 (t, J=8.2 Hz, aromatic 4-H), 8.20 (d, 2H, J=8.1 Hz, aromatic 3,5-H). MS (FAB) *m/z*: 388 (M^-). *Anal.* Calcd for $C_{14}H_{16}N_2O_{11}$: C, 43.31; H, 4.15; N, 7.21. Found: C, 43.09; H, 4.28; N, 7.03.

Potassium (2,4-Dinitrobenzyl β-D-Glucopyranosid)uronate (6a) A solution of **5a** (1.0 g, 2.58 mmol) in MeOH (200 ml) was treated with 0.4% K_2CO_3 (100 ml) in portions in an ice bath. The mixture was stirred at room temperature for 1 h, neutralized with Dowex 50W × 8 (H^+ form),

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filtered, and evaporated *in vacuo*. The residue was recrystallized from MeOH–acetone–H_2O (10 : 1 : 0.1) to give **6b** (colorless needles, decomp. 184—190 °C, 0.36 g, 33%). $[\alpha]_D^{20}$ −56.6° (c=0.1, H_2O). UV λ_{max} (H_2O) nm (ε): 249 (41900). IR (KBr) cm^{-1}: 3320 (OH), 1605 (COOK), 1541 (NO_2). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 3.14—3.26 (m, 4H, glucuronate 2,3,4,5-H), 4.38 (d, 1H, J=7.5 Hz, glucuronate 1-H), 5.18 (abq, 2H, J=17.0 Hz, methylene), 8.34 (d, 1H, J=8.5 Hz, aromatic 6-H), 8.59 (dd, 1H, J=2.4, 8.6 Hz, aromatic 5-H), 8.80 (d, 1H, J=2.4 Hz, aromatic 3-H). High-resolution MS m/z: Calcd for $C_{13}H_{14}N_2O_{11}K$: 413.0235. Found: 413.0234. *Anal.* Calcd for $C_{13}H_{13}N_2O_{11}K \cdot 0.5H_2O$: C, 37.06; H, 3.35; N, 6.65. Found: C, 37.34; H, 3.30; N, 6.63.

Potassium (2,6-Dinitrobenzyl β-D-Glucopyranosid)uronate (6b) Compound **5b** (1.0 g, 2.58 mmol) was treated with 0.4% K_2CO_3 (100 ml) in MeOH (200 ml) in the same manner as described for the synthesis of **5a**. After neutralization with Dowex 50W × 8 (H^+ form), the mixture was filtered and concentrated *in vacuo*. The residue was recrystallized from MeOH–acetone–H_2O (10 : 1 : 0.1) to give **6b** (colorless needles, decomp. 184—190 °C, 0.37 g, 34%). $[\alpha]_D^{20}$ −106° (c=0.1, H_2O). UV λ_{max} (H_2O) nm (ε): 236 (10600). IR (KBr) cm^{-1}: 3400 (OH), 1618 (COOK), 1541 (NO_2). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 2.84—3.24 (m, 4H, glucuronate 2,3,4,5-H), 4.14 (d, 1H, J=7.7 Hz, glucuronate 1-H), 4.98 (abq, 2H, J=13.0 Hz, methylene), 7.83 (t, 1H, J=8.1 Hz, aromatic 4-H), 8.23 (d, 2H, J=8.1 Hz, aromatic 3,5-H). High-resolution MS m/z: Calcd for $C_{13}H_{14}N_2O_{11}K$: 413.0235. Found: 413.0230. *Anal.* Calcd for $C_{13}H_{13}N_2O_{11}K \cdot 0.5H_2O$: C, 37.06; H, 3.35; N, 6.65. Found: C, 37.34; H, 3.30; N, 6.63.

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