SYNTHESIS OF 6-DEOXY-D-ALTROSE USED AS AN AUTHENTIC SAMPLE TO IDENTIFY AN UNKNOWN MONOSACCHARIDE ISOLATED FROM THE FRUITING BODY OF AN EDIBLE MUSHROOM

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Abstract – Here, we describe the synthesis of 6-deoxy-D-altrose and its subsequent use as an authentic sample to verify the structure of a monosaccharide newly isolated from the fruiting body of an edible mushroom. D-Rhamnopyranoside, converted from D-mannopyranoside, was selectively protected to give the 3-OH derivative, which was converted to the corresponding 6-deoxy-D-altropyranoside by nucleophilic substitution of the 3-triflate with acetoxy group. Removal of the protecting group, including the temporary protection of the anomeric position with the THP group, afforded the desired 6-deoxy-D-altrose. Both the NMR data and the \([\alpha]_D\) value were identical to the data on the natural product, thus indicating that the recently isolated monosaccharide was 6-deoxy-D-altrose.

Deoxy sugars are a common structural motif in biologically active natural products. Among the deoxy sugars, the 6-deoxy sugars are most prevalent, followed by sugars that are deoxygenated at the C-2 and C-3 positions.

6-Deoxy-altrose was first isolated from chemically reduced hygromycin, and later from the glycoprotein of fish eggs and the lipopolysaccharides of some bacteria. Although the authentic sample has been used to identify a component of glycoconjugates, there has been no report on the chemical synthesis of 6-deoxy-altrose to date. In the present study, we describe the efficient synthesis of 6-deoxy-D-altrose and then use it as an authentic sample to identify the unknown monosaccharide that we previously isolated from the fruiting body of an edible mushroom (Lactarius lividatus).

Dedicated to Prof. Dr. Albert Eschenmoser on the occasion of his 85th birthday.
To our knowledge, there is no commercial supply of 6-deoxy-D-altrose. Furthermore, the reported chemical synthesis affords only a partially protected derivative.  

Retrosynthetically, we employed D-rhamnoside 1 as a key intermediate, which was synthesized from D-mannose according to the procedure reported by Roy et al.  

The selective 3-O-benzylation of 1 was accomplished by the stannylation method, in which unprotected 1 was activated with dibutyltin oxide, benzylated in the presence of benzyl bromide and tetrabutylammonium iodide, then acetylated to afford 3-O-benzylated 3 in 79% yield over two steps. The deprotection of 3, with no acetyl migration, was accomplished in 98% yield by hydrogenolysis over palladium on carbon at atmospheric pressure. Next, 3-O-triflation of 4 was achieved using triflic anhydride and pyridine in dichloromethane to give 5, which was treated with tetrabutylammonium acetate in toluene to afford the expected 6-deoxy-D-altropyranoside 6 in 71% over two steps. The 1H NMR data of 6 confirmed the desired structure with the signal at 5.17 (dd, 1 Hz, J2,3 = 2.0 Hz, J3,4 = 3.5 Hz, H-3). A dramatic change in the J3,4 value from 9.6 Hz of 5 to 3.5 Hz of 6 indicated that the inversion of the configuration occurred at C-3. The oxidative removal of the p-methoxyphenyl group of per-O-acetylated 6-deoxy-D-altropyranoside 6 with ceric ammonium nitrate (CAN) afforded hemiacetal 7 in 68% yield, which was tentatively protected with the tetrahydropyranyl (THP) group to give 8 quantitatively. The removal of the acetyl group of 8 under Zemplen conditions, followed by acid hydrolysis of the THP group, gave the desired free 6-deoxy-D-altrose 10 in 78% over two steps. The NMR data and the [α]D values of the synthesized product were identical to those of the natural product, thus indicating that the monosaccharide recently isolated from the mushroom was 6-deoxy-D-altrose. The detailed comparison between the synthesized compound and the natural one will be published elsewhere.

![Scheme 1. Synthesis of 6-deoxy-D-altrose](image)

Reagents and conditions: (a) i) Bu2SnO/toluene, reflux, ii) BnBr, Bu4NI/toluene, reflux. (b) Ac2O, DMAP/py, rt. (c) H2, Pd(OH)2/EtOH. (d) Ti(OEt)4/py, CH2Cl2, 0°C. (e) Bu3NOAc/toluene, 85°C. (f) CAN/MeCN, toluene, H2O, rt. (g) DHP, PPTS/CH2Cl2. (h) NaOMe/MeOH, rt. (i) 2M-HCl
In conclusion, we accomplished an efficient synthesis of 6-deoxy-D-altrose. By using the synthesized compound as an authentic sample, the newly isolated and previously unknown monosaccharide from the edible mushroom (*Lactarius lividatus*) was confirmed to be that of 6-deoxy-D-altrose.

**EXPERIMENTAL**

General methods: Thin layer chromatography (TLC) was conducted on a Merck silica gel 60 F254 glass plate (Merck). Compounds were visualized under UV illumination at 254 nm or by spraying with a 10% H$_2$SO$_4$ in ethanol solution. Column chromatography on 80 mesh silica gel (Fuji Silysia Co.) was performed with the specified solvent system (v/v). Specific rotation was measured on a Horiba SEPA-300 high-sensitivity polarimeter at 25 °C. $^1$H NMR and $^{13}$C NMR spectra were recorded at 300 K on a Varian Inova 600/500 spectrometer, respectively. Values in ppm are given in reference to Me$_4$Si (in CDCl$_3$) or HOD (in D$_2$O, $\delta$ = 4.80) as the internal standard. High-resolution mass spectrometry (HRMS) was performed on a Bruker Daltonic microTOF (ESI-TOF) mass spectrometer. Molecular sieves were dried at 200 °C for 3 h in a muffle furnace prior to use. Solvents used as reaction media were dried over molecular sieves and used without further purification.

**4-Methoxyphenyl 3-O-benzyl-α-D-rhamnopyranoside (2):** Dibutyltin oxide (609 mg, 2.2 mmol) was added to a solution of 1 (520 mg, 1.83 mmol) in toluene (100 mL), and the mixture was refluxed for 20 h using a Dean-Stark trap, and cooled to rt. The mixture was treated with BnBr (267 µL, 2.2 mmol) and Bu$_4$NBr (709 mg, 2.2 mmol) under reflux for 3 h, and then concentrated. Column chromatography (1:6 EtOAc–hexane) of the residue on silica gel afforded 2 (547 mg, 79%) as crystals; [α]$_D$ +80.6° (c 1.0, CHCl$_3$). $^1$H NMR (CDCl$_3$): $\delta$ 1.28 (d, 3 H, J$_{5,6}$ = 6.4 Hz, H-6), 3.63 (t, 1 H, J$_{3,4}$ = J$_{4,5}$ = 9.6 Hz, H-4), 3.77 (s, 3 H, OMe), 3.80-3.86 (m, 2 H, H-3 and H-5), 4.21 (dd, 1 H, J$_{1,2}$ = 1.8 Hz, J$_{2,3}$ = 3.6 Hz, H-2), 4.66 and 4.78 (2 d, 2 H, PhCH$_2$), 5.44 (d, 1 H, H-1), 6.83 and 6.90 (2 d, 4 H, Ar), 7.35-7.41 (m, 5 H, Ph). $^{13}$C NMR(CDCl$_3$): $\delta$ 17.6, 55.6, 67.8, 68.4, 71.5, 71.8, 79.6, 98.2, 114.6, 117.6, 127.9, 128.7, 137.6, 150.2, and 154.9. HRMS: m/z: calcd for C$_{20}$H$_{24}$O$_6$+Na$: 383.1490 [M+Na]$^+$; found 383.1485.

**4-Methoxyphenyl 2,4-di-O-acetyl-3-O-benzyl-α-D-rhamnopyranoside (3):** Acetic anhydride (1.0 mL, 10 mmol) and 4-dimethylaminopyridine (DMAP; 20 mg, 0.16 mmol) were added to a solution of 2 (1.00 g, 2.66 mmol) in pyridine (27 mL) at 0 °C. The mixture was stirred for 1.5 h at rt. Completion of the reaction was confirmed by TLC (1:2 EtOAc–hexane). The reaction mixture was then diluted with EtOAc, and the organic layer was washed with 2 M HCl, sat. aq. NaHCO$_3$, and brine successively, then dried over Na$_2$SO$_4$, and concentrated. Column chromatography (1:7 EtOAc–hexane) of the residue on silica gel afforded 3 (1.21 g, 99%) as crystals; [α]$_D$ +17.2° (c 1.0, CHCl$_3$). $^1$H NMR (CDCl$_3$): $\delta$ 1.18 (d, 3 H, J$_{5,6}$ =
6.4 Hz, H-6), 2.03 and 2.17 (2 s, 6 H, 2 Ac), 3.77 (s, 3 H, OMe), 3.91 (m, 1 H, H-3), 4.50 and 4.71 (2 d, 2 H, PhCH₂), 5.10 (t, 1 H, J₃,₄ = J₄,₅ = 9.6 Hz, H-4), 5.35 (d, 1 H, J₁,₂ = 1.8 Hz, H-1), 5.52 (dd, 1 H, J₂,₃ = 3.2 Hz, H-2), 6.83 and 6.97 (2 d, 4 H, Ar), 7.29-7.35 (m, 5 H, Ph).

$^{13}$C NMR (CDCl₃): δ 17.4, 20.8, 20.9, 55.5, 67.1, 68.3, 71.3, 72.2, 74.3, 96.7, 114.5, 117.6, 127.6, 128.2, 137.8, 149.9, 155.1, 169.8, 170.2.


4-Methoxyphenyl 2,4-di-O-acetyl-α-D-rhamnopyranoside (4): 20% Pd(OH)₂ on activated carbon (3 g) was added to a solution of compound 3 (3.10 g, 6.73 mmol) in 1,4-dioxane and the suspension was stirred under a hydrogen atmosphere for 3 h at rt. After completion of the reaction was indicated by TLC (1:2 EtOAc–hexane), the reaction mixture was filtered through Celite and the filtrate was concentrated. Column chromatography (1:2 EtOAc–hexane) of the residue on silica gel afforded 4 (2.92 g, 98%) as crystals; [α]₀ +57.5º (c 1.0, CHCl₃).

$^1$H NMR (CDCl₃): δ 1.20 (d, 3 H, J₅,₆ = 6.2 Hz, H-6), 2.14 and 2.19 (2 s, 6 H, 2 Ac), 2.33 (broad d, 1 H, OH), 3.77 (s, 3 H, OMe), 3.97 (m, 1 H, H-5), 4.23 (m, 1 H, H-3), 4.97 (t, 1 H, J₃,₄ = J₄,₅ = 9.6 Hz, H-4), 5.24 (dd, 1 H, J₁,₂ = 1.4 Hz, J₂,₃ = 2.3 Hz, H-2), 5.40 (d, 1 H, H-1), 6.83 and 6.97 (2 d, 4 H, Ar).

$^{13}$C NMR (CDCl₃): δ 17.4, 21.0, 55.6, 66.6, 68.4, 72.6, 74.6, 94.2, 114.6, 117.6, 150.0, 155.2, 170.52, and 171.5. HRMS: $m/z$: calcd for C₁₇H₂₂O₈+: 377.1212 [M+Na]⁺; found 377.1218.

4-Methoxyphenyl 2,4-di-O-acetyl-3-O-trifluoromethanesulfonyl-α-D-rhamnopyranoside (5): Pyridine (111 µL, 1.37 mmol) and a solution of 4 (126 mg, 0.343 mmol) in CH₂Cl₂ were added dropwise at 0 °C to a solution of triflic anhydride (115 µL, 0.67 mmol) in CH₂Cl₂, and the mixture was stirred for 1 h at 0 °C. Completion of the reaction was confirmed by TLC (1:1 EtOAc–hexane). The reaction mixture was diluted with CHCl₃ and the organic layer was washed with 2 M HCl, sat. aq. NaHCO₃, water, and brine successively, then dried over Na₂SO₄, and concentrated. Column chromatography of the residue (1:8 EtOAc–hexane) on silica gel afforded 5 as a crude material, which was used in the next reaction without further purification. MS: $m/z$ (MALDI): calcd for C₁₈H₂₁O₁₀S+Na⁺: 509.07 [M+Na]⁺; found 509.05.

4-Methoxyphenyl 2,3,4-tri-O-acetyl-6-deoxy-α-D-altropyranoside (6): Anhydrous Bu₄NOAc (530 mg, 1.72 mmol) was added to a solution of 5 obtained above in toluene (25 mL), and the mixture was stirred for 1 h at 85 °C. Completion of the reaction was confirmed by TLC (1:4 EtOAc–hexane). The reaction mixture was concentrated after being cooled. Column chromatography (1:7 EtOAc–hexane) of the residue on silica gel afforded 6 (100 mg, 71% in two steps) as crystals; [α]₀ +107.5º (c 0.9, CHCl₃).

$^1$H NMR (CDCl₃): δ 1.21 (d, 3 H, J₅,₆ = 6.4 Hz, H-6), 2.06, 2.16, 2.16 (3 s, 3 H, 3 Ac), 3.78 (s, 3 H, OMe), 4.34-4.39 (m, 1 H, H-5), 5.01 (dd, 1 H, J₅,₄ = 3.5 Hz, J₄,₅ = 8.9 Hz, H-4), 5.17 (dd, 1 H, J₂,₃ = 2.0 Hz, H-3),
5.27-5.28 (m, 2 H, H-1,2), 6.83, and 6.97 (2 d, 4 H, Ar). $^{13}$C NMR (CDCl$_3$): $\delta$ 17.0, 20.6, 20.7, 55.4, 63.2, 67.1, 69.3, 69.6, 96.0, 114.4, 117.7, 150.2, 154.9, 169.2, 169.7, and 169.9. HRMS: $m/z$: calcd for C$_{19}$H$_{24}$O$_9$+Na$: 419.1318 [M+Na]$^+$: found 419.1315.

2,3,4-Tri-O-acetyl-6-deoxy-$\alpha$-D-altropyranoside (7): CAN (1.31 g, 2.39 mmol) and water (3 mL) were added to a solution of 6 (98 mg, 0.24 mmol) in toluene (5 mL) and MeCN (6 mL), and the mixture was stirred for 1 h at rt. Completion of the reaction was confirmed by TLC (1:1 EtOAc–hexane). The reaction mixture was then diluted with CHCl$_3$, and the organic layer was washed with water, sat. aq. NaHCO$_3$, and brine, then dried over Na$_2$SO$_4$, and concentrated. Column chromatography (1:8 EtOAc–hexane) of the residue on silica gel afforded 7 (67 mg, 92%) as an anomeric mixture: $^1$H NMR (CDCl$_3$): $\delta$ 5.38 (t, $J_{2,3}$ =$J_{3,4}$ =3.45 Hz, H-3b), $d$ 5.29 (t, $J_{2,3}$ =$J_{3,4}$ =3.45 Hz, H -3a), 5.19 (d, $J_{1,2}$ =6.85 Hz, H-1b), 5.03 (d, $J_{1,2}$ =3.40 Hz, H-1$^\alpha$), 4.99-4.94 (m, H -2$^\alpha$, H -2$^\beta$, H-4$^\alpha$), 4.83 (dd, $J_{4,5}$ =9.15 Hz, H-4$^\beta$), 4.34 (m, $J_{5,6}$ =6.40 Hz, H-5$^\alpha$), 4.00 (m, $J_{5,6}$ =6.40 Hz, H-5$^\beta$), 2.20, 2.13, 2.12, 2.06, 2.05, 2.02 (6 s, Ac), 1.25 (d, H-6). $^{13}$C NMR (CDCl$_3$): $\delta$ 170.1, 169.9, 169.8, 169.8, 169.7, 169.2, 91.7, 91.3, 70.1, 70.0, 69.8, 68.4, 67.3, 67.0, 63.8, 20.7, 20.6, 20.6, 20.5, 20.1, 17.6, 16.8.

Tetrahydropyranyl 2,3,4-tri-O-acetyl-6-deoxy-\(\alpha\)-D-altropyranoside (8): Pyridinium \(p\)-toluenesulfonate (PPTS; 38 mg, 0.151 mmol) and 3,4-dihydro-2H-pyran (214 $\mu$L, 2.27 mmol) were added to a solution of 7 (460 mg, 1.51 mmol) in CH$_2$Cl$_2$ (30 mL) under Ar atmosphere, and the mixture was stirred for 8 h at rt. Completion of the reaction was confirmed by TLC (1:1 ETOAc–toluene). After concentration, the residue was diluted with CHCl$_3$, and the organic layer was washed with brine, dried over Na$_2$SO$_4$, and concentrated. Column chromatography (1:3 EtOAc–hexane) of the residue on silica gel afforded 8 (586 mg, 100%) .

6-Deoxy-D-altrose (10): 28% NaOMe in MeOH (28 mL, 0.139 mmol) was added to a solution of 8 (540 mg, 1.39 mmol) in MeOH (30 mL), and the mixture was stirred for 0.5 h at rt. After monitoring the reaction by TLC (10:1 CHCl$_3$–MeOH), the reaction mixture was neutralized with Dowex (H$^+$). The resin was removed by filtration, and the filtrate was concentrated. The residue was treated with 2 M-HCl followed by neutralization with NaHCO$_3$ (330 mg). The reaction mixture was chromatographed on a column of Sephadex LH-20 (MeOH) to give the desired compound 10 (178 mg, 78%) as a mixture of \(\alpha\)-furanose (14%), \(\beta\)-furanose (10%), \(\alpha\)-pyranose (32%) and \(\beta\)-pyranose (44%); [\(\alpha\)]$_D$ +21° (c 1.0, after equilibrium in H$_2$O for 24 h). $^1$H NMR (D$_2$O): $\delta$ 5.29 (d, H-1$^\beta$), 5.25 (d, H-1$^\alpha$), 5.09 (d, H-1$^\beta$), 4.93 (d, H-1$^{\alpha}$), 4.20-3.69 (m), 3.56 (dd), 3.36 (s), 2.93 (t), 1.31-1.22 (m). $^{13}$C NMR (D$_2$O) d 101.0 (C-1$^\alpha$), 95.0 (C-1$^\beta$), 94.2 (C-1$^{\alpha}$), 92.6 (C-1$^{\beta}$), 87.3, 85.5, 82.6, 77.7, 76.4, 75.3, 72.1, 71.8, 71.6, 71.3, 70.9,
70.7, 70.4, 69.1, 68.7, 67.9, 18.4, 18.3, 18.2, 17.0. HRMS: m/z: calcd for C_{6}H_{12}O_{5}Na^{+}: 187.0582 [M+Na]^{+}: found 187.0579.

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