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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.

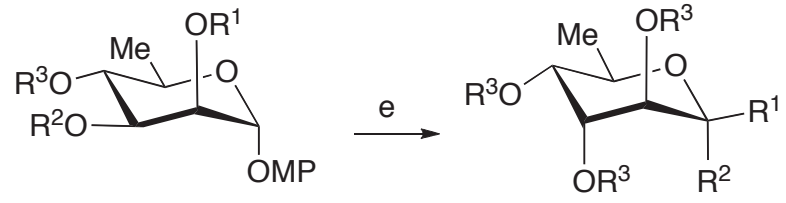
Deoxy sugars are a common structural motif in biologically active natural products. Among the deoxy sugars, the 6-deoxy sugars are most prevalent, followed by sugars that are deoxygenated at the C-2 and C-3 positions.

6-Deoxy-altrose was first isolated from chemically reduced hygromycin,[1] and later from the glycoprotein of fish eggs[2] and the lipopolysaccharides of some bacteria.[3,4] 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*).[5]

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.[6]

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*.[6] 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 ^{1}H NMR data of **6** confirmed the desired structure with the signal at 5.17 (dd, 1 Hz, $J_{2,3}$ = 2.0 Hz, $J_{3,4}$ = 3.5 Hz, H-3). A dramatic change in the $J_{3,4}$ 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 $[\alpha]_D$ 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.



	R^1	R^2	R^3	
1	H	H	H	a
2	H	Bn	H	b
3	Ac	Bn	Ac	c
4	Ac	H	Ac	d
5	Ac	Tf	Ac	

	R^1	R^2	R^3	
6	H	OMP	Ac	f
7	H, OH		Ac	g
8	H, OTHP		Ac	h
9	H, OTHP		H	i
10	H, OH		H	

Scheme 1. Synthesis of 6-deoxy-D-altrose
Reagents and conditiones: (a) i) Bu_2SnO/toluene, reflux, ii) BnBr, Bu_4NI/toluene, reflux. (b) Ac_2O, DMAP/py, rt. (c) H_2, $Pd(OH)_2$/EtOH. (d) Tf_2O/py, CH_2Cl_2, 0°C. (e) Bu_4NOAc/toluene, 85°C. (f) CAN/MeCN, toluene, H_2O, rt. (g) DHP, PPTS/CH_2Cl_2. (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_2SO_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_4Si (in $CDCl_3$) or HOD (in D_2O, δ = 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_4NBr (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; $[\alpha]_D$ +80.6° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 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($CDCl_3$): δ 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_2SO_4, and concentrated. Column chromatography (1:7 EtOAc–hexane) of the residue on silica gel afforded **3** (1.21 g, 99%) as crystals; $[\alpha]_D$ +17.2° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 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, PhCH_2), 5.10 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4), 5.35 (d, 1 H, $J_{1,2}$ = 1.8 Hz, H-1), 5.52 (dd, 1 H, $J_{2,3}$ = 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 ($CDCl_3$): 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, 127.6, 128.2, 137.8, 149.9, 155.1, 169.8, 170.2. MS: *m/z* (MALDI): calcd for $C_{24}H_{28}O_8$+Na^+: 467.17 $[M+Na]^+$: found 467.39.

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; $[\alpha]_D$ +57.5° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.20 (d, 3 H, $J_{5,6}$ 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, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4), 5.24 (dd, 1 H, $J_{1,2}$ = 1.4 Hz, $J_{2,3}$ = 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 ($CDCl_3$): 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 $C_{17}H_{22}O_8$+Na^+: 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 CH_2Cl_2 were added dropwise at 0 °C to a solution of triflic anhydride (115 μL, 0.67 mmol) in CH_2Cl_2, 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 $CHCl_3$ and the organic layer was washed with 2 M HCl, sat. aq. $NaHCO_3$, water, and brine successively, then dried over Na_2SO_4, 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 $C_{18}H_{21}O_{10}S$+Na^+: 509.07 $[M+Na]^+$ found 509.05.

4-Methoxyphenyl 2,3,4-tri-*O*-acetyl-6-deoxy-α-D-altropyranoside (**6**): Anhydrous Bu_4NOAc (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 ºC. 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; $[\alpha]_D$ +107.5° (*c* 0.9, $CHCl_3$), ^{1}H NMR ($CDCl_3$): δ 1.21 (d, 3 H, $J_{5,6}$ = 6.4 Hz, H-6), 2.06, 2.16, 2.16 (3 s, 9 H, 3 Ac), 3.78 (s, 3 H, OMe), 4.34-4.39 (m, 1 H, H-5), 5.01 (dd, 1 H, $J_{3,4}$ = 3.5 Hz, $J_{4,5}$ = 8.9 Hz, H-4), 5.17 (dd, 1 H, $J_{2,3}$ = 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 ($CDCl_3$): δ 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-a-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_2SO_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 ($CDCl_3$): d 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-1α), 4.99-4.94 (m, H-2α, H-2β, H-4α), 4.83 (dd, $J_{4,5}$ =9.15 Hz, H-4β), 4.34 (m, $J_{5,6}$ =6.40 Hz, H-5α), 4.00 (m, $J_{5,6}$ =6.40 Hz, H-5β), 2.20, 2.13, 2.12, 2.06, 2.05, 2.02 (6 s, Ac), 1.25 (d, H-6). ^{13}C NMR ($CDCl_3$): δ 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, 20.7, 20.6, 20.6, 20.5, 20.1, 17.6, 16.8.

Tetrahydropyranyl 2,3,4-tri-*O*-acetyl-6-deoxy-α-D-altropyranoside (**8**): Pyridinium *p*-toluenesulfonate (PPTS; 38 mg, 0.151 mmol) and 3,4-dihydro-2H-pyran (214 μL, 2.27 mmol) were added to a solution of **7** (460 mg, 1.51 mmol) in CH_2Cl_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_2SO_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 α-furanose (14%), β-furanose (10%), α-pyranose (32%) and β-pyranose (44)%; $[\alpha]_D$ +21° (*c* 1.0, after equilibrium in H_2O for 24 h),). ^{1}H NMR (D_2O): δ 5.29 (d, H-1*f*β), 5.25 (d, H-1*f*α), 5.09 (d, H-1*p*β), 4.93 (d, H-1*p*α), 4.20-3.69 (m), 3.56 (dd), 3.36 (s), 2.93 (t), 1.31-1.22 (m). ^{13}C NMR (D_2O) d 101.0 (C-1*f*α), 95.0 (C-1*f*β), 94.2 (C-1*p*α), 92.6 (C-1*p*β), 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 $C_6H_{12}O_5$+Na^+: 187.0582 $[M+Na]^+$: found 187.0579.

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