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Note

Synthesis of 2,5-anhydro-D-glucitol 6-phosphate*†

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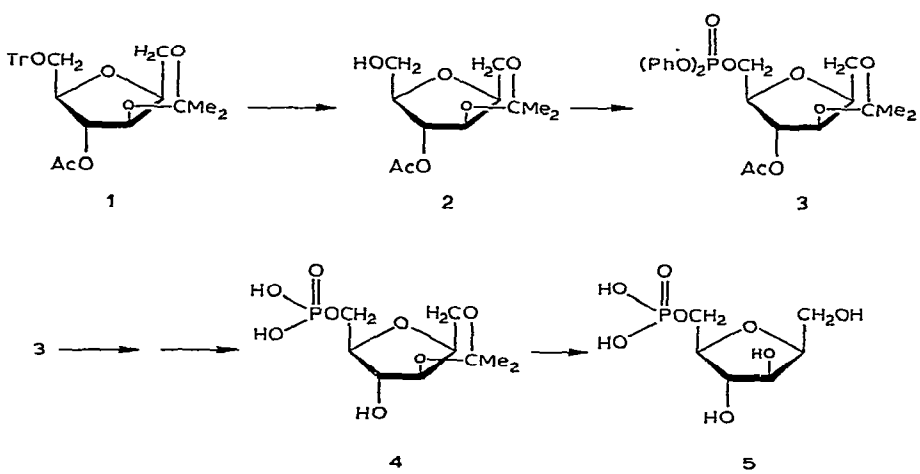
We have previously prepared 2,5-anhydro-D-glucitol 6-phosphate (**5**) enzymically[2] and shown it to be an effective inhibitor[2,3] of the enzyme phosphofructokinase (EC 2.7.1.11). However, enzymic phosphorylation at O-6 of 2,5-anhydro-D-glucitol was presumptive, and authentic **5** was needed for comparison with, and proof of structure of, the enzymically prepared compound. We now report an unambiguous, chemical synthesis of **5** from 4-*O*-acetyl-2,5-anhydro-1,3-*O*-isopropylidene-6-*O*-trityl-D-glucitol (**1**), a precursor whose synthesis from D-mannitol we have reported previously[1].



*New Derivatives of 2,5-Anhydro-D-hexitols. Part II. For Part I, see ref. 1.

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Catalytic hydrogenolysis of the trityl group from **1** yielded the appropriately protected intermediate, 4-*O*-acetyl-2,5-anhydro-1,3-*O*-isopropylidene-D-glucitol (**2**). This intermediate was phosphorylated at O-6 by the action of diphenylphosphorochloridate[4,5] in pyridine. Catalytic hydrogenolysis of the (diphenylphosphoryl)ated adduct (**3**) yielded the free acid, which could be simultaneously deacetylated and precipitated as its barium salt (**4**) through the action of methanolic barium methoxide. Acid hydrolysis of **4** removed the isopropylidene group and afforded the desired product, 2,5-anhydro-D-glucitol 6-phosphate (**5**), isolated as its crystalline di(cyclohexylammonium) salt. The optical rotation, i.r. spectrum, R_F on paper, and enzymic activity of **5** were identical with those of the enzymically prepared compound[2], proving the structure of the latter.

The overall yield of **5** was 30%, based on **1**. The major loss occurred in the initial, detritylation step. Examination of the product of this step by t.l.c. and ^{1}H-n.m.r. spectroscopy showed appreciable (~50%) hydrolysis of the isopropylidene protecting group. This side reaction did not seem to be due to the presence of a trace of hydrochloric acid in the palladium–charcoal catalyst, as neither repeated washing of the catalyst nor addition of barium carbonate to the reaction mixture improved the yield. However, the contamination of **2** with de-isopropylidenated material posed no problem, as the impurity could be quantitatively removed from a chloroform solution of the reaction mixture by aqueous extraction, as demonstrated by performing the extraction in an n.m.r. tube and monitoring the process *in situ* by ^{1}H-n.m.r. spectroscopy.

The (diphenylphosphoryl)ated adduct **3** was obtained as a crystalline solid, and was thoroughly characterized. Its ^{1}H-n.m.r. spectrum yielded all vicinal-proton coupling-constants, as well as the two expected ^{1}H–^{31}P couplings and a long-range coupling ($^4J_{2,4}$ 0.5 Hz). Based on the coupling-constant data and arguments presented previously[1] for **1** and related compounds, the probable conformation of the tetrahydrofuran ring of **3** is E_1(D).

EXPERIMENTAL

General methods. — For details of general synthetic and analytical procedures, see[1] Part I. Ascending paper-chromatography, not previously described, was conducted on Whatman No. 40 filter paper for the diammonium salt of **5** by use of 3:2 (v/v) isopropyl ether–88% formic acid as the solvent, and development with acid-molybdate spray[6]. Catalytic hydrogenolyses were performed in a Parr, shaker-type, hydrogenation apparatus. Elemental analyses were made by Galbraith Laboratories, Inc., Knoxville, Tenn.

4-O-Acetyl-2,5-anhydro-1,3-O-isopropylidene-D-glucitol (**2**). — A solution of[1] **1** (4.09 g, 8.37 mmol) in absolute ethanol (280 ml) was hydrogenolyzed at a pressure of one atmosphere in the presence of 10% palladium-on-charcoal (3.0 g; prewashed with absolute ethanol). After the uptake of 9.0 mmol (106%) of hydrogen, the reaction was complete (~80 min). The catalyst was removed by filtration, and the

filtrate evaporated *in vacuo*. The semisolid residue was then triturated, and extracted six times, with 25-ml portions of hexane to remove triphenylmethane. The syrup remaining was dissolved in chloroform (75 ml), and the solution was washed twice with aqueous sodium hydrogencarbonate (75 ml). The chloroform layer and a 50-ml chloroform back-extract of the aqueous layer were combined, dried (anhydrous sodium sulfate), and evaporated *in vacuo*. The resulting, straw-colored syrup was dried *in vacuo*, to yield **2** (957 mg, 47%) that was homogeneous by t.l.c. (R_F 0.29, diethyl ether); ^{1}H-n.m.r. data (chloroform-*d*, Me_4Si; 100 MHz): δ 1.38 and 1.44 (2 s, 3 H each, isopropylidene methyls), 2.08 (s, 3 H, acetate methyl), ~2.8 (D_2O-exchangeable, s, 1 H, hydroxyl proton), 3.85 (1 H, X of ABX, A of AMX, $J_{1,2}$ 2.5 Hz, $J_{1',2}$ 2.0 Hz, $J_{2,3}$ 2.8 Hz, H-2), 3.8–3.9 (m, 2 H, H-6,6'), ~4.0 (m, 1 H, $J_{4,5}$ 2.0 Hz, H-5), 4.06 (1 H, A of ABX, $J_{1',1}$ <0.1 Hz, $J_{1',2}$ 2.0 Hz, H-1'), 4.07 (1 H, B of ABX, $J_{1,1'}$ <0.1 Hz, $J_{1,2}$ 2.5 Hz, H-1), 4.29 (1 H, M of AMX, $J_{2,3}$ 2.8 Hz, $J_{3,4}$ 0.5 Hz, H-3), and 4.95 (1 H, X of AMX, $J_{3,4}$ 0.5 Hz, $J_{4,5}$ 2.0 Hz, H-4).

Anal. Calc. for $C_{11}H_{18}O_6$ (246.26): C, 53.65; H, 7.37. Found: C, 53.21; H, 7.46.

*4-O-Acetyl-2,5-anhydro-1,3-O-isopropylidene-*D*-glucitol 6-(diphenylphosphate)* (**3**). — To a well-stirred solution of **2** (957 mg, 3.9 mmol) in dry pyridine (10 ml) at 0° was added diphenylphosphorochloridate (1.80 ml, 8.2 mmol). After 40 min at 0° and 68 h at 4°, t.l.c. showed the reaction to be complete. Unreacted reagent was then decomposed by adding water (2 ml) and stirring for 1 h. The mixture was now evaporated *in vacuo* at 45° to a viscous oil; this was dissolved in chloroform (100 ml), and the solution was washed once with ice-cold, 0.5M sulfuric acid (50 ml) and once with saturated sodium hydrogencarbonate solution (50 ml). The chloroform layer and a chloroform back-extract (10 ml) were combined, dried (anhydrous sodium sulfate), and evaporated *in vacuo*. The oily product was dissolved in ethanol (50 ml), and water was added to faint turbidity. After storage at 4°, a white solid formed; this was recrystallized from chloroform–hexane, yielding short, colorless plates of **3** (1.62 g, 87%), m.p. 83.1–83.4°, $[\alpha]_D^{20}$ +18.90 ±0.13° (*c* 2.0, chloroform); R_F 0.38 (diethyl ether); λ_{max} 5.71 (C=O of acetate), 7.23 and 7.28 (d, CMe_2), 7.80 P=O of phosphoric triester), 8.10–8.55 (m, C–O of POPh), 9.28–9.88 (m, C–O of $POCH_2R$), 10.30–10.70 (m, P–O of POPh), 12.63, 12.73, 12.85, and 14.40 μm (all four, Ph); ^{1}H-n.m.r. data (chloroform-*d*, Me_4Si; 100 MHz): δ 1.31 and 1.35 (2s, 3 H each, isopropylidene methyls), 1.99 (s, 3 H, acetate methyl), 3.84 (1 H, X of ABX, A of AMRX, $J_{1,2}$ 2.8 Hz, $J_{1',2}$ 1.5 Hz, $J_{2,3}$ 2.8 Hz, $^4J_{2,4}$ 0.5 Hz, H-2), 3.95 (1 H, A of ABX, $J_{1,1'}$ <0.1 Hz, $J_{1',2}$ 1.5 Hz, H-1'), 3.96 (1 H, B of ABX, $J_{1,1'}$ <0.1 Hz, $J_{1,2}$ 2.8 Hz, H-1), 4.14 (1 H, X of AMRX, X of second ABX, $J_{4,5}$ 1.5 Hz, $J_{5,6}$ 7.3 Hz, $J_{5,6'}$ 5.5 Hz, H-5), 4.22 (1 H, M of AMRX, $J_{2,3}$ 2.8 Hz, $J_{3,4}$ 0.5 Hz, H-3), 4.44 (1 H, A of second ABX, $J_{5,6'}$ 5.5 Hz, $J_{6,6'}$ <0.1 Hz, $J_{6',P}$ 7.5 Hz, H-6'), 4.45 (1 H, B of second ABX, $J_{5,6}$ 7.3 Hz, $J_{6,6'}$ <0.1 Hz, $J_{6,P}$ 7.5 Hz, H-6), 4.96 (1 H, R of AMRX, $J_{3,4}$ 0.5 Hz, $J_{4,5}$ 1.5 Hz, $^4J_{2,4}$ 0.5 Hz, H-4) and 7.00–7.45 (m, 10 H, phenyl protons).

Anal. Calc. for $C_{23}H_{27}O_9P$ (478.44): C, 57.74; H, 5.69; P, 6.47. Found: C, 57.38; H, 5.72; P, 6.61.

2,5-Anhydro-D-glucitol 6-phosphate, di(cyclohexylammonium) salt (**5**). — A solution of **3** (1.17 g, 2.45 mmol) in absolute methanol (100 ml) was hydrogenolyzed at a pressure of one atmosphere in the presence of platinum oxide (250 mg, 2.3 mmol). After the uptake of 22 mmol (100%) of hydrogen, the reaction was complete (1 h). The catalyst was removed by filtration, and the acid in the filtrate was neutralized (pH 9) with freshly prepared and filtered barium methoxide solution (~80mM). The resulting, clear gel was evaporated *in vacuo* to a viscous syrup that crystallized when allowed to become hydrated, yielding 1.10 g of **4** (99%). Washed Dowex-50W X8 (H^+) cation-exchange resin (100 meq. suspended in ~40 ml of water) was added to a solution of **4** (1.01 g, 2.22 mmol) in water (10 ml). The resulting suspension was stirred for 40 min at room temperature, gently boiled under reflux for 5 min, quickly cooled to room temperature, and filtered, first through a sintered-glass plate and then through washed Celite. The acid in the clear filtrate was neutralized with cyclohexylamine to pH 7.0, and the solution was evaporated *in vacuo*. The pH of solution of the resulting, viscous syrup in water (10 ml) was adjusted to 10.5 with cyclohexylamine, and the solution was slowly diluted with acetone (150 ml). After being kept overnight at 4°, crystals of the salt of **5** were deposited; yield 753 mg (74%; 30% overall, based on **1**), m.p. 134.5–136.6°, $[\alpha]_D^{20}$ +7.79 ±0.06° (*c* 2, water).

Anal. Calc. for $C_{18}H_{39}N_2O_8P \cdot H_2O$ (460.51): C, 46.95; H, 8.97; N, 6.08; P, 6.73. Found: C, 46.81; H, 8.97; N, 6.05; P, 6.73.

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