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Note

Synthesis of D-arabinose 5-phosphate and D-xylose 5-phosphate

HUBERT MAEHR AND JOANNE SMALLHEER
Chemical Research Department, Hoffmann-La Roche Inc , Nutley, New Jersey 07110 (U.S.A.)
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The present interest in pentose 5-phosphates reaches beyond the members of the phosphogluconate pathway and has recently centered on D-arabinose 5-phosphate (**5a**). D-Arabinose is utilized by certain yeasts[1,2], presumably[3] *via* **5a**, and by bacteria[3,4]. In *Aerobacter aerogenes*, D-arabinose is isomerized to D-*erythro*-pentulose, followed by phosphorylation at O-5 and epimerization at C-3 to produce D-xylose 5-phosphate[4]. *Pseudomonas saccharophila* metabolizes D-arabinose *via* D-arabinofuranose → D-arabinono-1,4-lactone → D-arabinonic acid → 3-deoxy-D-*glycero*-2-pentulosonic acid → pyruvic acid and glycolic acid[5]. The catabolism of D-arabinose in *Escherichia coli* K-12 involves the sequence D-arabinose → D-*erythro*-pentulose → D-*erythro*-pentulose 1-phosphate → 1,3-dihydroxy-2-propanone 1-phosphate and glycolaldehyde[6], whereas, in *E. coli* B/r, the pathway D-arabinose → D-*erythro*-pentulose → D-*erythro*-pentulose 5-phosphate → D-*threo*-pentulose 5-phosphate has been established[7].

Compound **5a** is an intermediate in the pathway of cell-wall synthesis in Gram-negative bacteria. Condensation of **5a** (available, *inter alia*, from D-*erythro*-pentulose 5-phosphate *via* the action of D-*erythro*- pentulose 5-phosphate isomerase[8]) with enolpyruvate phosphate leads to 3-deoxy-D-*manno*-octulosonic acid 8-phosphate[9], which is a constituent of the cell-wall lipopolysaccharide. Consonant with this pathway was the isolation of an auxotrophic mutant of *Salmonella typhimurium* having impaired ability to synthesize the 3-deoxy-D-*manno*-octulosonate region of the lipopolysaccharide, and dependent on exogenous **5a** for growth[10]. It has been suspected that D-*erythro*-pentulose 5-phosphate isomerase is generally present in, and limited to, those bacteria wherein D-arabinose is a major structural element within the polysaccharide chains, as in *Mycobacterium tuberculosis, Mycobacterium leprae*, some corynebacteria, and Nocardia[8]. The limitation of this enzyme system to bacteria was thought to have possible therapeutic significance[8]. To evaluate this hypothesis, a ready access to **5a** was required that none of the published methods seem to provide.

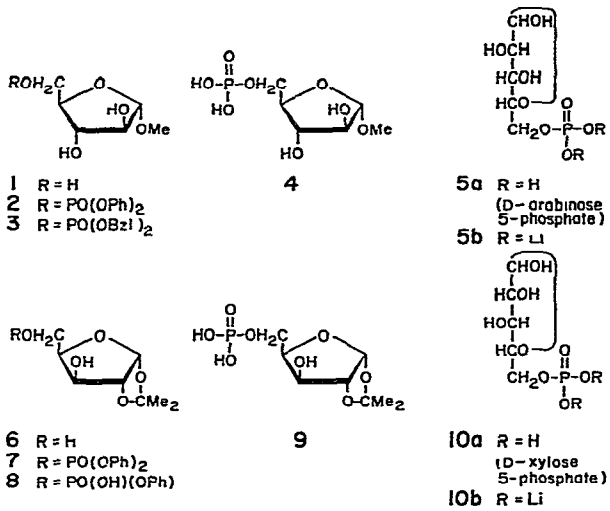
Starting with D-arabinose, the first synthesis of **5a** involved 7 steps[11], and the

products derived either from direct phosphorylation of D-arabinose or oxidation of D-glucose 6-phosphate by lead tetraacetate appear to lack characterization[9]. A preparation of **5a** obtained by ninhydrin degradation of 2-amino-2-deoxy-D-glucose 6-phosphate[12] was shown to be contaminated with D-*erythro*-pentulose 5-phosphate[8], and the procedure of Szabó *et al.*[3], starting with D-glucose, comprises 9 steps, with unspecified overall yield. The most recently published synthesis of L-arabinose 5-phosphate[13] from L-arabinose, although adaptable to the preparation of **5a**, is not attractive, in view of the 8 steps involved.

Selective phosphorylation of the 5'-hydroxyl group of unprotected nucleosides has been actively studied, and accomplished with many reagents[14-16]. Similar selective phosphorylation was obtained with other substrates[17], so that economical syntheses of pentose 5-phosphates appeared possible by phosphorylation of a suitably protected pentofuranose with such readily accessible phosphorylating reagents as diphenyl phosphorochloridate[18] or dibenzyl phosphorochloridate[19], followed by hydrogenolytic generation of the furanose 5-phosphate derivative, and autohydrolysis. We illustrate this approach by the synthesis of the 5-phosphates of D-arabinose and D-xylose.

Methyl α-D-arabinofuranoside (**1**), prepared from D-arabinose dipropyl dithioacetal, was phosphorylated with an equivalent of diphenyl phosphorochloridate. The resulting ester (**2**) was purified by chromatography, and hydrogenolyzed with hydrogen in the presence of platinum oxide to give methyl α-D-arabinofuranoside 5-phosphate (**4**). The use of diphenyl phosphorochloridate has the advantage of stability and commercial availability of the reagent; hydrogenolytic removal of the phenyl groups is reportedly only possible with platinum catalysts[18,19]; we have confirmed this observation, and find palladium-on-carbon to be ineffective. It is surprising, therefore, that successful hydrogenolysis of 1,2,3-tri-*O*-acetyl-5-*O*-(diphenylphosphono)-L-arabinofuranose apparently succeeded with palladium chloride in ethanol[13]. Dibenzyl phosphorochloridate is a rather unstable reagent, and must be prepared immediately before use. The phosphorylation yields with this reagent are generally low, presumably due in part to instability of the product, but the benzyl groups undergo hydrogenolysis with particular ease. Alternatively, therefore, ester **4** was also prepared from methyl 5-*O*-(dibenzylphosphono)-α-D-arabinofuranoside (**3**) by hydrogenolysis with palladium-on-carbon; autohydrolysis of **4**, derived from either **2** or **3**, was performed without prior isolation, and yielded **5a**, isolated as the dilithium salt (**5b**).

D-Xylose 5-phosphate was prepared analogously. 1,2-*O*-Isopropylidene-α-D-xylofuranose (**6**) was phosphorylated with diphenyl phosphorochloridate to give crystalline 5-*O*-(diphenylphosphono)-1,2-*O*-isopropylidene-α-D-xylofuranose (**7**) in good yield. Hydrogenolysis of **7**, however, proved to be somewhat unpredictable. In one experiment, 1,2-*O*-isopropylidene-α-D-xylofuranose 5-phosphate (**9**) was obtained in 1 h with platinum oxide in ethanol and a hydrogen pressure of 3.4 atm.; in another, similar conditions led to the partially hydrogenolyzed and crystalline ester **8**. At 204 atm, hydrogenolysis was accomplished without formation of **8** as



a byproduct. Autohydrolysis of ester **9** gave D-xylose 5-phosphate (**10a**) readily isolated as the dilithium salt (**10b**).

EXPERIMENTAL

General methods. — All evaporations were performed under diminished pressure. Methanol was redistilled from magnesium methoxide, and pyridine from powdered potassium hydroxide. Melting points are uncorrected. ^{1}H-N.m.r. spectra were recorded with a Varian HA-100 spectrometer. T.l.c. was conducted on plates of silica gel 60 F254 (E. Merck, Darmstadt) with systems *A* 8:1 (v/v) chloroform–methanol, *B* 9:1 (v/v) butanone–water, and *C* 10:5:1 (v/v) methanol–chloroform–pyridine. Spots were detected under u.v. light or with a sulfuric acid spray and subsequent charring. Chromatographic columns were prepared by slurrying silicic acid (Mallinckrodt, 100 mesh) in chloroform.

Methyl α-D-arabinofuranoside[20] (**1**). — To a mechanically stirred, ice-cold suspension of D-arabinose dipropyl dithioacetal[21] (5.69 g, 20 mmol) and powdered Drierite (3 g) in methanol (110 ml) were added mercuric chloride (10.9 g) and yellow mercuric oxide (5 g) in one portion. Stirring was continued for 2 h in an ice bath, and for 30 min at room temperature. The suspension was filtered, the solids were thoroughly washed with methanol, and the filtrate and washings were combined, diluted with pyridine (5 ml), and cooled in an ice bath for 1 h. The pyridine–$HgCl_2$ complex was removed by filtration, and the filtrate evaporated to dryness; the residue was dissolved in water (30 ml), the solution was cooled in an ice bath for 1 h, and the suspension was filtered. The filtrate was evaporated to a syrup which was dried under diminished pressure, to yield **1** (3.12 g, 19 mmol); R_F 0.18 (*A*) and 0.50 (*B*); ^{1}H-n.m.r. data ($CDCl_3$): δ 4.86 (s, H-1, $J_{1,2} \leq 1$ Hz).

Methyl 5-O-(diphenylphosphono)-α-D-arabinofuranoside (**2**). — Crude **1** (2.89 g,

17.6 mmol) as obtained in the previous experiment was dissolved in pyridine (30 ml), and the solution was evaporated to remove moisture. The residue was dissolved in pyridine (10 ml), the solution was cooled to 0°, and a solution of diphenyl phosphorochloridate[22] (5.05 g, 18.8 mmol) in dichloromethane (30 ml) was added with stirring during 1.5 h at 0°. Stirring at ice-bath temperature was continued for a further 3 h. Water (25 ml) was then added, the mixture was stirred for 30 min at room temperature, and the phases were separated. The organic phase was washed with water (2 × 25 ml), and evaporated to dryness. The residue was dissolved in chloroform (20 ml) and placed on top of a column of silicic acid (61 g). Chloroform (350 ml) eluted minor impurities; the major component was now eluted with 9:1 chloroform–ethyl acetate (400 ml) and then solvent *A* (150 ml). The chromatographically pure fractions (t.l.c.) were pooled, and evaporated to yield **2** as a syrup (3.64 g, 8.64 mmol; 49%) $[\alpha]_D^{25}$ +49° (*c* 0.5, ethanol); R_F 0.44 (*A*), 0.83 (*B*), and 0.86 (*C*); ^{1}H-n.m.r. data ($CDCl_3$): δ 2.20 (s, 0.5 H, CH_3OH), 3.28 (s, CH_3O), 3.33 (s, 0.5 CH_3OH), 3.77–4.38 (m, 7 H, H-2–5 and 2 OH), 4.73 (s, H-1), and 7.21 (m, 10 H, 2 C_6H_5).

Anal. Calc. for $C_{18}H_{21}O_8P \cdot 0.5\ CH_3OH \cdot 0.5\ H_2O$ (421.37): C, 52.73; H, 5.74; P, 7.35. Found: C, 52.60; H, 5.41; P, 7.61.

Methyl 5-O-(dibenzylphosphono)-α-D-arabinofuranoside (**3**). — *N*-Chlorosuccinimide (2.8 g) was added to a solution of dibenzyl phosphite (5.32 g, 20 mmol) in benzene (50 ml), and the mixture was stirred for 2 h and filtered[23]. The filtrate was evaporated, the residue dissolved in dichloromethane (10 ml), and the solution added dropwise during 30 min to a solution of anhydrous **1** (3.12 g, 19 mmol) in 2,6-dimethylpyridine (5 ml) and acetonitrile (10 ml) at ice-bath temperature. The temperature was then allowed to rise to room temperature, but a considerable quantity of starting material (t.l.c., *B*) still remained. Therefore, the addition of crude dibenzyl phosphorochloridate was repeated at room temperature. After 4 h, chloroform (20 ml) was added, and the mixture was successively washed with water (2 × 20 ml), saturated sodium hydrogencarbonate solution (30 ml), and water (20 ml), and evaporated. To aid removal of 2,6-dimethylpyridine, toluene was repeatedly added to, and evaporated from, the residue. The residue was dissolved in chloroform, and chromatographed on a column of silicic acid (35 g). Chloroform (400 ml) eluted impurities; the major product was obtained with 97:3 chloroform–2-propanol, affording, after removal of solvent, pure **3** as a colorless syrup (2.9 g, 6.83 mmol; 34%); R_F 0.55 (*A*), 0.60 (*B*), and 0.52 (*C*); ^{1}H-n.m.r. data ($CDCl_3$): δ 3.35 (s, OCH_3), 3.66 (s, b, 2 OH), 3.93–4.29 (m, 5 H, H-2–5), 4.84 (s, H-1), and 5.01 and 5.03 (d, 2 H each, $J_{H,P}$ 8 Hz, 2 $OCH_2C_6H_5$).

D-*Arabinose 5-(dilithium phosphate)* (**5b**) from **3** or **2**. — A solution of **3** (650 mg, 1.53 mmol) in 2-propanol (20 ml) was hydrogenolyzed at a hydrogen pressure of 3.4 atm. in the presence of 10% palladium-on-carbon (50 mg) for 2.5 h, to yield **4** (R_F 0.2, *C*). The catalyst was removed by filtration, the filtrate evaporated to dryness, the residue dissolved in water (~1 ml), and the solution heated under nitrogen on a steam bath until hydrolysis was complete. The resulting solution of

5a (R_F 0.1, *C*) was decolorized with activated carbon, and passed through a column of Dowex 50-X4 (H^+) ion-exchange resin (200–400 mesh, 2 ml). The effluent and aqueous washings were combined, adjusted to pH 8.1 with M lithium hydroxide, and concentrated to ~0.8 ml. Addition of methanol and acetone gave a white precipitate that was successively washed with ethanol and ether, to yield **5b** as the monohydrate (310 mg, 1.19 mmol; 78%), $[\alpha]_D^{25}$ +11.2° (*c* 1.0, 0.1M HCl); lit. $[\alpha]_D^{22}$ +10°, +13° (ref. 3, for the sesquihydrate), $[\alpha]_D^{20}$ −12.8° for the[13] L-isomer (monohydrate).

Anal. Calc. for $C_5H_9Li_2O_8P \cdot H_2O$ (259.99): C, 23.10; H, 4.26; P, 11.91. Found: C, 23.17; H, 4.29; P, 12.18.

Hydrogenolysis of **2** (3.40 g, 8.07 mmol) in methanol (30 ml) with hydrogen at a pressure of 3.4 atm. in the presence of platinum oxide (200 mg) was complete in 2 h. The catalyst was removed by filtration, the filtrate containing **4** was evaporated, the residue was dissolved in water (2 ml), and the solution was heated, under nitrogen, on a steam bath until hydrolysis was complete. The light-tan solution was decolorized with activated carbon, and the acid converted into its dilithium salt as already described. The aqueous solution (8 ml) was added to ethanol (100 ml), and the precipitate was collected by centrifugation, washed with ethanol, and dried, to yield **5b** as its monohydrate (1.77 g, 6.81 mmol; 84%), having the same properties as that from **3**.

5-O-(Diphenylphosphono)-1,2-O-isopropylidene-α-D-xylofuranose (**7**). — A solution of **6** (ref. 24) (3.80 g, 20 mmol) in pyridine (25 ml) was evaporated to remove moisture, the residue was redissolved in pyridine (13 ml), the solution was cooled in an ice bath, and a solution of diphenyl phosphorochloridate[22] (5.94 g, 21 mmol) in dichloromethane (20 ml) was added dropwise with stirring during 90 min. Stirring at ice-bath temperature was continued for 2 h, when starting-material (R_F 0.45, *A*) was no longer detectable. The mixture was diluted with dichloromethane (35 ml), and washed with water (3 × 10 ml). The organic phase was evaporated, and the product crystallized immediately. To complete the crystallization, hexane was added, and the mixture was refrigerated overnight. The crystals were collected, washed with hexane, and recrystallized from chloroform–hexane, to yield **7** (7.1 g, 16.8 mmol; 84%), m.p. 103–104°; $[\alpha]_D^{25}$ −10.8° (*c* 0.5, ethanol); R_F 0.81 (*A*), 0.90 (*B*), and 0.89 (*C*); ^{1}H-n.m.r. data ($CDCl_3$): δ 1.26 (s, CH_3), 1.44 (s, CH_3), 3.66 (s, b, OH), 4.02–4.39 (m, 4 H, H-3–5), 4.46 (d, H-2, $J_{1,2}$ 3.4 Hz), 5.85 (d, H-1, $J_{1,2}$ 3.4 Hz)[25], and 7.21 (m, 10 H, 2 C_6H_5).

Anal. Calc. for $C_{20}H_{23}O_8P$ (422.37): C, 56.87; H, 5.49. Found: C, 57.18; H, 5.52.

1,2-O-Isopropylidene-α-D-xylofuranose 5-(phenyl hydrogenphosphate) (**8**). — A suspension of platinum oxide (100 mg) in ethanol (2 ml) was prehydrogenated, a solution of **7** (1 g, 2.37 mmol) in ethanol (5 ml) was added, and the mixture was stirred for 2 h with hydrogen at a pressure of 3.4 atm. The catalyst was removed by filtration, and the filtrate and washings (ethanol) were combined and evaporated. The residue was crystallized from 2-propanol–ether (604 mg, 1.70 mmol, 72%), and

recrystallized from 2-propanol, to give **8**, m.p. 148–150°; R_F 0.74 (*C*); ^{1}H-n.m.r. data (Me_2SO-d_6): δ 1.24 (s, CH_3), 1.37 (s, CH_3), 3.83–4.32 (m, 4 H, H-3–5), 4.39 (d, H-2, $J_{1,2}$ 3.5 Hz), 5.85 (d, H-1, $J_{1,2}$ 3.5 Hz), and 7.06–7.56 (m, 7 H, C_6H_5, P-OH, 0.5 H_2O).

Anal. Calc. for $C_{14}H_{19}O_8P \cdot 0.5\ H_2O$ (355.29): C, 47.33; H, 5.67. Found: C, 46.90; H, 5.44.

In a repetition of this experiment, supposedly under essentially the same conditions, **9** was formed exclusively.

D-*Xylose 5-(dilithium phosphate)* (**10b**). — A mixture of **7** (1 g, 2.37 mmol), platinum oxide (0.5 g), and ethanol (10 ml) was shaken for 24 h with hydrogen at a pressure of 204 atm. The catalyst was removed by filtration, and the filtrate was evaporated to give **9** as a syrup (R_F 0.31, *C*) which was dissolved in water (1 ml); the solution was heated on a steam bath, under nitrogen, for 1 h, by which time, no starting material was detectable. The light-tan solution of **10a**, R_F 0.09 (*C*), was diluted with water, decolorized with activated carbon, the suspension filtered and the filtrate adjusted to pH 8.5 with 0.1M lithium hydroxide solution. The solution was filtered again, the filtrate concentrated to a small volume, and twenty volumes of ethanol were added. The resulting precipitate was collected by centrifugation, successively washed with ethanol and ether by resuspension and recentrifugation, and dried, to yield **10b** (400 mg, 1.52 mmol; 64%), $[\alpha]_D^{25}$ +10.8° (*c* 0.9, water), lit.[26] $[\alpha]_D^{22}$ +5° (barium salt).

Anal. Calc. for $C_5H_9Li_2O_8P \cdot 0.5\ H_2O \cdot 0.25\ C_2H_5OH$ (262.50): C, 25.17; H, 4.42; P, 11.80. Found: C, 25.39; H, 4.42; P, 11.79.

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