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SYNTHESIS OF 5,6-DIDEOXY-6-PHOSPHONO-D-*arabino*-HEXOSE, AN ISOSTERIC PHOSPHONATE ANALOGUE OF D-ARABINOSE 5-PHOSPHATE

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ABSTRACT

5,6-Dideoxy-6-phosphono-D-*arabino*-hexose, an isosteric phosphonate analogue of D-arabinose 5-phosphate, was synthesized in six steps from the known methyl 5-*O*-triphenylmethyl-α-D-arabinofuranoside. The key step involves pyridinium chlorochromate oxidation of methyl 2,3-di-*O*-acetyl-α-D-arabinofuranoside to the 5-*aldehydo* derivative, and Wittig synthesis with (diphenyl phosphonomethylene)triphenylphosphorane. 5,6-Dideoxy-6-phosphono-D-*arabino*-hexose, a compound of interest for the study of Gram-negative lipopolysaccharide biosynthesis, is converted by 3-deoxyoctulosonate 8-phosphate synthetase from *Escherichia coli* K 235 into a material yielding a thiobarbituric acid chromophore with λ_{max} 550 nm, probably 9-phosphono-3,8,9-trideoxy-D-*manno*-nonulosonate, an isosteric phosphonate analog of 3-deoxyoctulosonate 8-phosphate (KDO 8-phosphate).

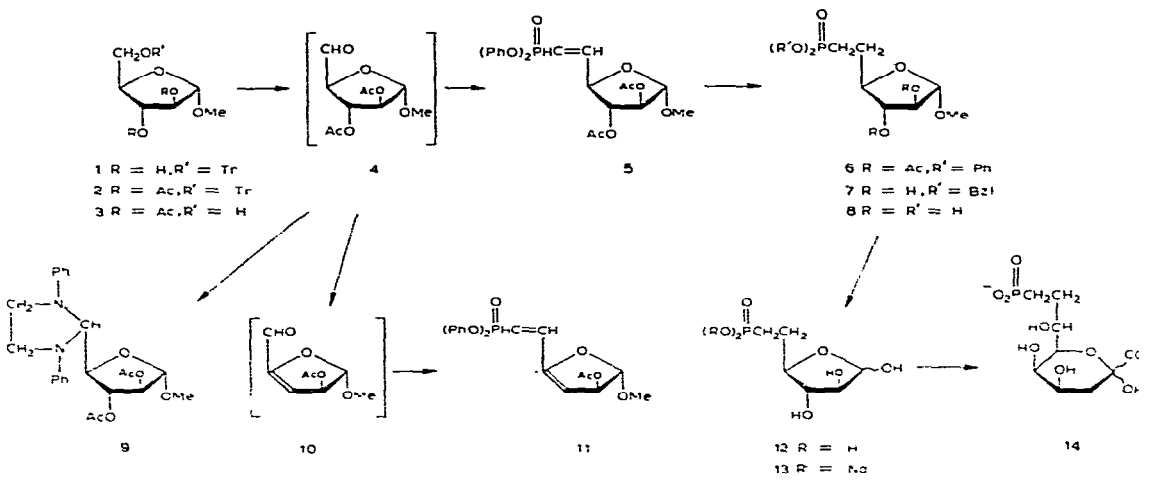
INTRODUCTION

In a search for inhibitors of the biosynthesis of the Gram-negative bacterial lipopolysaccharide (LPS), we are studying the biosynthesis and cell-surface incorporation of 3-deoxy-D-*manno*-octulosonic acid (KDO), a component present in all Gram-negative LPS structures investigated so far[1]. One step of KDO biosynthesis involves condensation of D-arabinose 5-phosphate with phosphoenol pyruvate, catalyzed by 3-deoxyoctulosonate 8-phosphate synthetase[2] (*1*). The 3-deoxyoctulosonate 8-phosphate (KDO 8-phosphate) formed is dephosphorylated by a specific phosphatase[3] to give KDO (*2*)

$$\text{D-Ara-5-P} + \text{phosphoenol pyruvate} \rightarrow \text{KDO-8-P} + P_i \qquad (1)$$

$$\text{KDO-8-P} \rightarrow \text{KDO} + P_i \qquad (2)$$

It was expected that 5,6-dideoxy-6-phosphono-D-*arabino*-hexose (**13**), an isosteric phosphonate analog of D-arabinose 5-phosphate, would be a substrate of reaction (*1*) so as to produce 3,8,9-trideoxy-9-phosphono-D-*manno*-nonulosonic acid (**14**), which would resist dephosphorylation in step (*2*) and possibly inhibit dephos-



phorylation of the natural substrate, KDO 8-phosphate. Since KDO 8-phosphate is not a substrate of the "activating" enzyme, CMP-3-deoxyoctulosonate synthetase[4], and since there appears to be an essential requirement for KDO in LPS biosynthesis[5], it was felt that such inhibition might provide a basis for the rational design of antibacterial agents.

DISCUSSION

The present synthesis of **13** constitutes an adaptation of an original phosphonate synthesis that Jones and Moffatt[6] developed for application to ribonucleosides and certain pentofuranosides. In this procedure, an unprotected hydroxymethyl group (at C-4 of a pentofuranoside or C-5′ of a nucleoside) is oxidized to an aldehyde group, which is condensed with crystalline (diphenylphosphonomethylene)triphenylphosphorane[7] to give a vinyl phosphonate. This, upon selective hydrogenation of the double bond, affords the saturated phosphonic acid ester. Methyl 5-*O*-triphenylmethyl-α-D-arabinofuranoside[8] (**1**) was acetylated and the resulting compound detritylated to afford the diacetate **3** in very good yields. Oxidation of **3** to the *aldehydo* derivative, **4**, was best carried out by use of the pyridinium chlorochromate reagent[9] in benzene solution. Several other methods were tested, such as Pfitzner–Moffatt oxidation with a variety of acidic catalysts[10] and oxidations with silver carbonate, *N*-chlorobenzotriazole, chromium trioxide-on-graphite, and pyridinium chromate[11], but gave inferior yields. The aldehyde **4** could not be isolated in the pure state by column chromatography on silicic acid. Compound **9**, a crystalline 1,3-diphenylimidazolidine derived from **4**, was obtained in 50% yield by the procedure used by Jones and Moffatt[10] for the analogous derivative of 2′,3′-*O*-cyclohexylideneuridine; release of **4** from **9** was accompanied, however, by extensive losses. It was, therefore, found more convenient to utilize the pyridinium chlorochromate reagent and to proceed directly to **5** as soon as the presence of maximal

amounts of **4** was indicated by t.l.c. The Wittig adduct **5**, obtained in crystalline form, showed an n.m.r. spectrum in excellent agreement with the values reported by Jones and Moffatt[6] for the corresponding vinyl phosphonate derived from uridine, thus suggesting a *trans* geometry of the vinyl group. Even under optimal conditions, pyridinium chlorochromate oxidation proceeded with some elimination to give the α,β-unsaturated aldehyde **10**. The corresponding Wittig adduct, **11**, was chromatographically isolated and identified by n.m.r. spectroscopy (see Experimental section). Formation of elimination products analogous to aldehyde **10** has been reported to occur in Pfitzner–Moffatt oxidations of appropriately protected nucleosides[12] and hexopyranosides[13]; the more basic dimethyl sulfoxide–sulfur trioxide–pyridine–triethylamine reagent was used in the latter work[13]. Catalytic hydrogenation of **5** with 10% palladium-on-barium sulfate gave **6** in quantitative yield. Treatment of **6** with sodium benzyl alcoholate by the procedure of Jones and Moffatt[6] gave, in good yield, the benzyl ester **7**, which was stable on prolonged storage in a desiccator. Hence, it was found convenient to prepare the free sugar phosphonate **13** in small batches from **7**, as required for enzyme experiments. Catalytic hydrogenation of **7** over palladium oxide in methanol yielded the unstable phosphonic acid **8**, which could be converted into its ammonium salt, but for practical purposes was immediately hydrolyzed to give the free sugar phosphonic acid **12**. This was immediately converted into its disodium salt (**13**), and then stored in small aliquots at −20°. The ^{1}H-n.m.r. spectrum of **13** gave little information on the structure, but the specific rotation and the behavior on electrophoresis, t.l.c., toward silver nitrate reagents, and as substrate of 3-deoxyoctulosonate 8-phosphate synthetase from *E. coli* K 235 (see Experimental section) are closely similar to those of the commercially available disodium D-arabinose 5-phosphate.

The formation of a compound reacting with thiobarbituric acid, probably free KDO, from phosphoenol pyruvate and increasing concentrations of D-arabinose

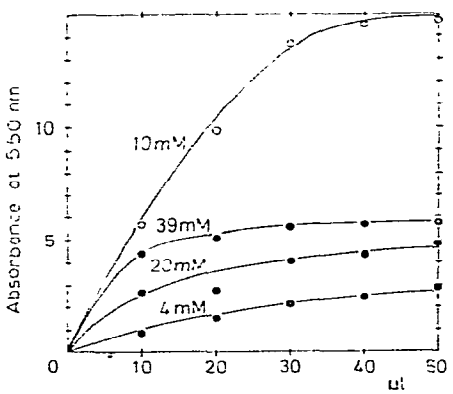


Fig. 1. Absorbance at 550 nm, obtained in the standard 3-deoxyoctulosonate 8-phosphate synthetase assay of Ghalambor and Heath[2], *vs.* µl of substrate solution used; ○, D-arabinose 5-disodium phosphate (10mM); ●, **13** (39mM, 20mM, and 4mM).

5-phosphate, catalyzed by 3-deoxyoctulosonate 8-phosphate synthetase, is shown in Fig. 1 (open circles). The product formation increases steadily with increasing concentrations of D-arabinose 5-phosphate. By contrast, increasing concentrations of **13** under identical conditions gave rise to a limited formation of a compound reacting with thiobarbituric acid, probably 3,8,9-trideoxy-9-phosphono-D-*manno*-nonulosonate (**14**, closed circles in Fig. 1). When **13** was the substrate, the initial rates were high, whereas a certain concentration of product seems to inhibit the reaction. As the 3-deoxyoctulosonate 8-phosphate synthetase present in the crude extracts used in this study is usually associated with 3-deoxyoctulosonate 8-phosphate phosphatase[3], it is probable that the enzymically produced KDO 8-phosphate was immediately dephosphorylated into KDO. Therefore, increasing concentrations of KDO were observed rather than of KDO 8-phosphate when D-arabinose 5-phosphate was the substrate (open circles in Fig. 1). With **13** as substrate, the enzymic synthesis of **14**, which is resistant to the physiological dephosphorylation mechanism, is expected and conceivably, the curves (closed circles in Fig. 1) represent the inhibition by **14** of product formation by 3-deoxyoctulosonate 8-phosphate synthetase. Previous experiments[14] have shown that product inhibition by KDO 8-phosphate can be observed with highly purified (phosphatase free) preparations of 3-deoxyoctulosonate 8-phosphate synthetase acting on D-arabinose 5-phosphate as the substrate.

EXPERIMENTAL

General. — Melting points were determined on a Kofler hot-stage and are uncorrected. Optical rotations were determined on a Perkin–Elmer 141 polarimeter. Elemental analyses were performed by Dr. J. Zak, Mikroanalytisches Laboratorium am Institut für Physikalische Chemie, Universität Wien. N.m.r. spectra were obtained on a Varian HA-100 instrument with tetramethylsilane as the internal standard: chemical shifts are reported in p.p.m. (δ) and signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), or m (complex multiplet); coupling constants are first order. Thin-layer chromatography (t.l.c.) was performed on Merck pre-coated plates (5 × 10 cm), layer thickness 0.35 mm, Silica gel 60 F_{254}. Compositions of solvent mixtures are indicated throughout by volume (v/v). Spots were detected by u.v. light and by spraying with an anisaldehyde–sulfuric acid reagent[15]. *N*-Chlorobenzotriazole was obtained from the Parish Chemical Co., Provo, Utah 84601 (USA) and chromium trioxide-on-graphite (Seloxcette) came from Alfa Products, Beverly, MA 01915 (USA).

Methyl 5-O-triphenylmethyl-α-D-arabinofuranoside (**1**). — This compound was prepared as described by Glaudemans and Fletcher[8] in 73% yield (lit. yield, 68%), m.p. 112–114° (from benzene–petroleum ether), $[\alpha]_D^{20}$ +92.9° (*c* 0.53, chloroform); lit.[8] m.p. 112–113° and $[\alpha]_D^{20}$ +62.4° (*c* 1.56, ethyl acetate); n.m.r. (chloroform-*d*): δ 2.83 (d, 1 H, J ~10 Hz, OH), 3.27 (d of d's, 1 H, $J_{5,5'}$ ~11 Hz, $J_{5,4}$ ~2.5 Hz, H-5), 3.40 (s, 3 H, CH_3O), 3.65 (d of d's, 1 H, $J_{5',5}$ ~11 Hz, $J_{5',4}$ ~3 Hz, H-5'), 3.75–4.20 (m, 4 H, H-2, H-3, H-4, OH), 5.00 (s, 1 H, H-1), and 7.20–7.55 (m, 15 H,

aromatic H of Tr); ν_{max}^{KBr} 3475 (OH), 3030 (CH arom.), 1600, 1498 (C=C arom. rings); u.v.: benzene absorptions.

Methyl 2,3-di-O-acetyl-5-O-triphenylmethyl-α-D-arabinofuranoside (**2**). — This compound was obtained in practically quantitative yield by standard acetylation (acetic anhydride–pyridine) of **1**; m.p. 110–112° (benzene–petroleum ether), $[\alpha]_D^{20}$ +47.0° (*c* 0.66, chloroform); n.m.r. (chloroform-*d*): δ 2.04, 2.07 (2 s, 6 H, AcO), 3.24–3.54 (m, 2 H, H-5,5'), 3.40 (s, 3 H, Me), 4.18 (d of d's of d's, 1 H, $J_{4,5} \sim J_{4,5'} \sim J_{4,3} \sim 5$ Hz, H-4), 4.96 (s, 1 H, H-1), 5.06 (d, 1 H, $J_{2,3} \sim 1.5$ Hz, H-2), 5.20 (d of d's, 1 H, $J_{3,2} \sim 1.5$ Hz, $J_{3,4} \sim 5$ Hz, H-3), and 7.10–7.60 (m, 15 H, arom. H of Tr); ν_{max}^{KBr} 3030 (CH arom.), 1720 (C=O esters), 1580, 1475 (C=C arom. rings), and 1215cm^{-1} (C–O esters); u.v.: benzene absorptions.

Anal. Calc. for $C_{28}H_{30}O_7$: C, 71.0; H, 6.1. Found: C, 70.9; H, 6.1.

Methyl 2,3-di-O-acetyl-α-D-arabinofuranoside (**3**). — Compound **2** (16 g, 32.6 mmol) was mixed with 80% acetic acid (250 ml) and boiled for 10 min under reflux. Upon cooling, some triphenylmethanol crystallized and was removed by filtration. The filtrate was neutralized with sodium hydrogencarbonate (294 g) in water (4 l), and extracted with chloroform (3 × 300 ml). The extract was dried (magnesium sulfate) and evaporated, and the resulting viscous solution applied to a column (4 × 80 cm, 450 g of Merck Silica gel 60 F_{254}) prepared in 1:1 benzene–ethyl acetate. After all remaining triphenylmethanol had been eluted with the same solvent, the column was washed with ethyl acetate to elute **3** as a clear, colorless syrup (6 g, 74%), $[\alpha]_D^{20}$ +63.3° (*c* 0.55, chloroform); n.m.r. (chloroform-*d*): δ 2.11 (s, 6 H, AcO), 2.75 (broad m, 1 H, OH), 3.40 (s, 3 H, Me), 3.75–3.90 (m, 2 H, H-5,5'), 4.11 (d of d's of d's, 1 H, $J_{4,5} \sim J_{4,5'} \sim J_{4,3} \sim 5$ Hz, H-4), 4.92 (s, 1 H, H-1), 5.03 (d of d's, 1 H, $J_{3,2} \sim 1.5$ Hz, $J_{3,4} \sim 4$ Hz, H-3), and 5.08 (d, 1 H, $J_{2,3} \sim 1.5$ Hz, H-2); $\nu_{max}^{CH_2Cl_2}$ 3625 (OH), 1740 (C=O ester), and 1220 cm^{-1} (C–O ester).

Anal. Calc. for $C_{10}H_{16}O_7$: C, 48.4; H, 6.5. Found: C, 48.7; H, 6.5.

Methyl 2,3-di-O-acetyl-5,6-dideoxy-6-(O,O-diphenyl)phosphono-α-D-arabino-hex-5-enofuranoside (**5**). — Compound **3** (3.5 g, 14 mmol) was dissolved in dry benzene (100 ml), pyridinium chlorochromate (3 g, 13.9 mmol; Fluka, Buchs, SG, Switzerland) was added, and the mixture stirred for 8 h at 50° with exclusion of moisture. After filtration over Celite, the filtrate was mixed with (diphenylphosphonomethylene)triphenylphosphorane[7] (3.5 g, 6.9 mmol) and stirred overnight at 50° with exclusion of moisture. The solvent was evaporated, the residue dissolved in 2:1 benzene–ethyl acetate, and the solution applied to a column (4 × 80 cm) of silica gel (450 g, Merck 60 F_{254}). Elution with the same solvent gave first phenol (R_F 0.72, 150 mg), and then **11** (R_F 0.57, 350 mg) identified[6,12] on the basis of the n.m.r. spectrum: δ 2.04 (s, 3 H, AcO), 3.51 (s, 3 H, CH_3O), 5.34 (s, 1 H, H-1), 5.40–5.60 (m, 2 H, H-2, H-3), 6.45 (d of d's, 1 H, $J_{6,5} \sim 17$ Hz, $J_{6,P} \sim 20$ Hz, H-6), and 6.80–7.50 (m, 11 H, H-5 and aromatic H's of phenyl groups[6]). Compound **5** (1.52 g, 23%; 50% if recovered **3** is taken into consideration) was eluted subsequently, R_F 0.4, $[\alpha]_D^{20}$ +31.9° (*c* 0.68, chloroform); crystallization from ether–light petroleum gave waxy, hygroscopic crystals, m.p. 52° (sealed tube); n.m.r.: δ 2.03, 2.10 (2 s, 6 H,

AcO), 3.42 (s, 3 H, CH_3O), 4.65 (d of d's of d's, 1 H, $J_{4,6}$ ~2 Hz, $J_{4,3}$ ~6 Hz, $J_{4,5}$ ~4 Hz, H-4), 4.84 (d of d's, 1 H, $J_{3,4}$ ~6 Hz, $J_{3,2}$ ~1.5 Hz, H-3), 5.01 (s, 1 H, H-1), 5.10 (d, 1 H, $J_{2,3}$ ~1.5 Hz, H-2), 6.36 (d of d's of d's, 1 H, $J_{6,5}$ ~17 Hz, $J_{6,P}$ ~21 Hz, $J_{6,4}$ ~2 Hz, H-6)[6], and 6.90–7.45 (m, 11 H, H-5 and aromatic H's of phenyl groups[6]); $\nu_{max}^{CHCl_3}$ 1745 (C=O esters), 1797, 1490 (C=C aromatic rings), and 1260–1190 cm^{-1} (P=O, C–O–P, and C–O esters); λ_{max}^{MeOH} 267 (ε 165), 261 (ε 182), and 256 nm (ε 165).

Anal. Calc. for $C_{23}H_{25}O_8P$: C, 58.0; H, 5.3; P, 6.5. Found: C, 58.8; H, 5.3; P, 6.7.

Further elution gave a 4th fraction (560 mg, R_F 0.31), probably containing a dimeric compound; n.m.r. spectrum showed 4 acetyl and 2 methoxyl groups; mol. wt. >461 (m.s.); its structure has not been established so far. Finally, unchanged **3** (1.97 g, R_F 0.2) was eluted.

*Methyl 2,3-di-*O*-acetyl-4-(1,3-diphenylimidazolidin-2-yl)-*α*-*D*-arabino-tetra-*O*-furanoside* (**9**). — This compound was prepared in 50% yield from **3**, essentially following the procedure described by Jones and Moffatt[10] for the preparation of the corresponding derivative of 2′,3′-*O*-cyclohexylideneuridine, m.p. 117–118° (from methanol), $[\alpha]_D^{20}$ +51.3° (*c* 0.45, chloroform); n.m.r. (chloroform-*d*): δ 1.71, 2.07 (2 s, 6 H, AcO), 3.32 (s, 3 H, CH_3O), 3.55–3.95 [m, 4 H, $-H(CH_2)_2N-$], 4.52 (d of d's, 1 H, $J_{4,3}$ ~5 Hz, $J_{4,5}$ ~1.5 Hz, H-4), 4.90 (s, 1 H, H-1), 4.93 (d, 1 H, $J_{2,3}$ ~1.5 Hz, H-2), 5.22 (d of d's, 1 H, $J_{3,4}$ ~5 Hz, $J_{3,2}$ ~1.5 Hz, H-3), 5.93 (d, 1 H, $J_{5,4}$ ~1.5 Hz, H-5), 6.65–6.95, and 7.15–7.40 (2 m's, 6 H+4 H, aromatic H's of diphenylethylenediamine moiety); ν_{max}^{KBr} 3030 (CH arom.), 1735 (C=O esters), 1593, 1498 (C=C arom. rings), and 1220 cm^{-1} (C–O esters); λ_{max}^{MeOH} 353 (ε 33 700) and 292 nm (ε 4490).

Anal. Calc. for $C_{24}H_{29}N_2O_6$: C, 65.5; H, 6.4; N, 6.4. Found: C, 65.6; H, 6.4; N, 6.4.

*Methyl 2,3-di-*O*-acetyl-5,6-dideoxy-6-(O,O-diphenyl)phosphono-*α*-*D*-arabino-hexofuranoside* (**6**). — This compound was prepared from **5** by catalytic hydrogenation in the presence of 10% palladium-on-barium sulfate in practically quantitative yield; the reaction was followed by t.l.c. (2:1 benzene–ethyl acetate), **5** having R_F 0.4 and **6** R_F 0.34; colorless syrup, $[\alpha]_D^{20}$ +37.7° (*c* 0.44, chloroform); n.m.r. (chloroform-*d*): δ 2.09 (s, 6 H, AcO), 2.10–2.45 (m, 4 H, H-5, H-6), 3.38 (s, 3 H, CH_3O), 4.15 (d of t's, 1 H, $J_{4,5}$ ~5 Hz, $J_{4,3}$ ~7 Hz, H-4), 4.89 (d of d's, 1 H, $J_{3,4}$ ~7 Hz, $J_{3,2}$ ~2 Hz, H-3), 4.90 (s, 1 H, H-1), 5.08 (d, 1 H, $J_{2,3}$ ~2 Hz, H-2), and 7.00–7.45 (m, 10 H, aromatic H's of phenyl groups); $\nu_{max}^{CHCl_3}$ 1745 (C=O esters), 1597, 1490 (C=C arom. rings), and 1250–1190 cm^{-1} (P=O, C–O–P, and C–O esters); λ_{max}^{MeOH} 268 (ε 1090), 263 (ε 1250), and 257 nm (ε 1040).

Anal. Calc. for $C_{23}H_{27}O_8P$: C, 57.7; H, 5.6; P, 6.5. Found: C, 57.1; H, 5.7; P, 6.3.

*Methyl 6-(O,O-dibenzyl)phosphono-5,6-dideoxy-*α*-*D*-arabino-hexofuranoside* (**7**). — Compound **6** (2.0 g, 4.2 mmol) was dissolved in dry benzyl alcohol (10 ml), and a solution of sodium (~0.8 g, 34.8 mmol) in dry benzyl alcohol (40 ml) was added.

After 2 h at room temperature with exclusion of moisture, t.l.c. (5:5:1 butanone–benzene–ethanol) showed that **6** (R_F 0.80) had disappeared with formation of **7** (R_F 0.40). The reaction mixture was neutralized with Dowex 50 (H^+, 20 ml, previously rinsed with benzyl alcohol) cation-exchange resin, and most of the benzyl alcohol removed by evaporation (oil pump). The syrupy residue was applied to a column (80 × 3 cm) of silica gel (Merck 60 F_{254}), chromatographed with the just described solvent mixture, and the fractions containing **7** were pooled to give 1.27 g (72%) of a colorless syrup, $[\alpha]_D^{20}$ +57.2° (*c* 0.36, chloroform); n.m.r. (chloroform-*d*): δ 1.73–2.25 (m, 4 H, J ~5–6 Hz, $-CH_2CH_2P-$), 3.29 (s, 3 H, CH_3CO), 3.73 (d of d's, 1 H, $J_{3,2}$ ~3.5 Hz, $J_{3,4}$ ~6 Hz, H-3), 3.90 (q, 1 H, $J_{4,3} \sim J_{4,5,5'} \sim 6$ Hz, H-4), 4.09 (d of d's, 1 H, $J_{2,1}$ ~1.5 Hz, $J_{2,3}$ ~3.5 Hz, H-2), 4.79 (d, 1 H, $J_{1,2}$ ~1.5 Hz, H-1), 4.95 (d, 4 H, J_{CH_2OP} ~8 Hz, aliphatic Bzl H), and 7.29 (s, 10 H, aromatic Bzl H); $\nu_{max}^{CHCl_3}$ 3375 (OH), 2835 (OCH_3), 1240–1180, 1090, and 1020–960 cm^{-1} (several bands, P=O and P–O–C); λ_{max}^{MeOH} 268 (ε 300), 263 (ε 435), 257 (ε 504), and 252 nm (ε 411).

Anal. Calc. for $C_{21}H_{27}O_7P$: C, 59.7; H, 6.4; P, 7.3. Found: C, 59.0; H, 6.5; P, 7.1.

5,6-Dideoxy-6-phosphono-D-arabino-*hexose, sodium salt* (**13**). — Compound **7** (300 mg, 0.71 mmol) was dissolved in dry methanol (20 ml) and hydrogenated at atmospheric pressure in the presence of palladium oxide (500 mg) overnight. The reaction was monitored by t.l.c. (5:5:1 butanone–benzene–ethanol) whereby **7** had R_F ~0.4 and **8** remained at the origin. The catalyst was filtered off over Celite and washed well with dry methanol, and the filtrate and washings were evaporated to give **8** (170 mg, 99%) as a clear, slightly pink syrup. It was dissolved in water (10 ml) and the solution was boiled with Dowex 50 (H^+) cation-exchange resin under a stream of argon for ~2 h, after which **8** (R_F ~0.43 in 3:2:3 butanol–acetic acid–water) had been converted into the free sugar **12** (R_F ~0.39). The resin was filtered off and the pH of the solution was adjusted to 7.0 with sodium hydroxide; lyophilization gave **13** (170 mg, 93% based on **7**; 25% based on **1**), $[\alpha]_D^{20}$ +12.0° (*c* 0.44, water; equilibrium); by comparison, commercial D-arabinose disodium 5-phosphate (Sigma Chemical Co., St. Louis, Missouri, U.S.A.) had $[\alpha]_D^{20}$ +7.4° (*c* 0.41, water, equilibrium). On t.l.c. (3:2:3 butanol–acetic acid–water), both commercial D-arabinose disodium 5-phosphate and **13** had R_F 0.39. On analytical paper electrophoresis (20 min, 2500 V, 35 mA; Whatman 3 MM; 989:10:1 water–pyridine–acetic acid, pH 6.9; total distance 42 cm), commercial D-arabinose disodium 5-phosphate travelled a distance of 14 cm and **13** moved 13 cm, both being detected with a silver nitrate spray[16]. Compound **13** has not been obtained crystalline so far; after lyophilization of its solution, a hygroscopic glass was obtained which did not give the required values on elemental analysis.

Formation of compounds reactive with thiobarbituric acid from **13** *and phosphoenol pyruvate in the presence of 3-deoxyoctulosonate 8-phosphate synthetase from* Escherichia coli *K 235.* — Crude extracts of *E. coli* K 235, used as the source of enzyme, were prepared essentially by the procedure described by Nirenberg[17] for *E. coli* W3100, except that the final ultracentrifugation was carried out at 200 000*g*.

The supernatant solutions (S-200) were satisfactory for 3-deoxyoctulosonate 8-phosphate synthetase reaction. A typical solution contained ~12 mg of protein[18] per ml and a specific activity of ~0.10 unit* of 3-deoxyoctulosonate 8-phosphate synthetase per mg of protein. The reaction mixture (in triplicate) contained M glycylglycine buffer (pH 7.2, 20 μl), 0.1M phosphoenol pyruvate (5 μl), 0.01M D-arabinose phosphate 5-disodium (10–50 μl) or various concentrations of **13** (10–50 μl, see legend to Fig. 1), S-200 (5 μl), and water for a total volume of 100 μl. The reactants were mixed at 0° and the mixtures incubated at 37° for 30 min. The concentration of compounds reacting with thiobarbituric acid (λ_{max} 550 nm) was measured by the Warren assay[19] as modified by Kean and Roseman[20].

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*One unit of 3-deoxyoctulosonate 8-phosphate synthetase is the amount of enzyme that catalyzes the turnover of 1 μmol of D-arabinose 5-phosphate per min (ref. 2).