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SYNTHESIS OF 3-DEOXY-D-*arabino*-2-HEPTULOSONIC ACID 7-PHOSPHATE BY PHOSPHORYLATION OF METHYL (METHYL 3-DEOXY-D-*arabino*-HEPTULOPYRANOSID)ONATE*

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ABSTRACT

3-Deoxy-D-*arabino*-2-heptulosonic acid 7-phosphate (**5**), the first committed intermediate in aromatic amino acid biosynthesis, has been synthesized in good yield by treatment of methyl (methyl 3-deoxy-D-*arabino*-2-heptulopyranosid)onate with diphenylphosphoric chloride under mild conditions to give the 7-diphenyl phosphate. Catalytic removal of the phenyl residues, followed by base-catalyzed hydrolysis resulted in formation of (methyl 3-deoxy-D-*arabino*-2-heptulopyranosid)onic acid dihydrogen 7-phosphate (**4**), which yielded a crystalline tris-(cyclohexylammonium) salt. Acid-catalyzed hydrolysis of **4** afforded **5**, which was used to purify 3-dehydroquinate synthase.

INTRODUCTION

3-Deoxy-D-*arabino*-2-heptulosonic acid 7-phosphate (**5**), formed in bacteria and plants from phosphoenolpyruvate and D-erythrose 4-phosphate, is the first committed intermediate in aromatic amino acid biosynthesis[1,2]. Relatively large amounts of **1** were required as substrate in the purification of 3-dehydroquinate synthase[3], and in other studies[4,5]. The availability of methyl (methyl 3-deoxy-D-*arabino*-2-heptulopyranosid)onate (**1**) by a previous synthesis[6] suggested its use in the synthesis of **5** by selective phosphorylation of the primary hydroxyl group with diphenylphosphoric chloride[7,8]. This approach appeared to be attractive since, in our earlier synthesis[9] of **5**, the final step, selective oxidation of a polyhydroxy acid to a polyhydroxy-2-keto acid with a mixture of vanadium pentaoxide and sodium chlorate, occasionally gave low yields.

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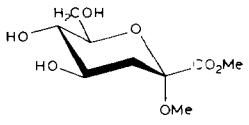
RESULTS AND DISCUSSION

Treatment of **1** with diphenylphosphoric chloride under mild conditions[8] yielded syrupy methyl (methyl 3-deoxy-D-*arabino*-2-heptulopyranosid)onate diphenyl 7-phosphate (**2**), which on catalytic hydrogenation gave syrupy methyl (methyl 3-deoxy-D-*arabino*-2-heptulopyranosid)onate dihydrogen 7-phosphate (**3**). Saponification of **3** yielded (methyl 3-deoxy-D-*arabino*-2-heptulopyranosid)onic acid dihydrogen 7-phosphate (**4**) which formed a crystalline tris (cyclohexylammonium) salt. Hydrolysis of **4** with dilute hydrochloric acid was incomplete and afforded **5**, isolated as a barium salt that was free from inorganic phosphate but contained a small proportion of **4**.



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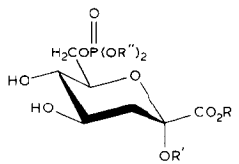
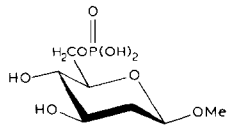


2 R = Me, R′ = Me, R″ = Ph
3 R = Me, R′ = Me, R″ = H
4 R = H, R′ = Me, R″ = H
5 R = H, R′ = H, R″ = H



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Thus, the synthesis of **5** from **1** was straightforward and furnished appreciable amounts of material suitable for enzymic studies[3–5], but slightly contaminated by the glycoside **4**. On the other hand, (methyl 2-deoxy-β-D-*arabino*-hexopyranoside) dihydrogen 6-phosphate (**6**) was completely hydrolyzed at 88mM concentration, by its own acidity, at 68° within 12 h without release of inorganic phosphate[8]. Compound **4**, in which a carboxyl group is substituted for H at C-1 relative to **6**, showed only a 56% glycoside hydrolysis under similar conditions (Table I). More glycoside hydrolysis occurred as heating was prolonged, but also a greater release of inorganic phosphate. In **4**, the positive charge of the carbonyl group dipole in CO_2H, in position β to O-6, may reduce the rate of protonation of O-2 (or of O-6) to an oxonium ion, and may, therefore, lower the rate of hydrolysis. A similar stabilization against acid hydrolysis was found in methyl 2-amino-2-deoxy-D-glucopyranoside, as compared to ethyl β-D-glucopyranoside[10]. The small proportion of **4** present in purified **5** did not affect its usefulness as a substrate for 3-dehydroquinate synthase, since its concentration in the assay was far below that required for inhibition[3].

The ^{1}H-n.m.r. spectrum of the methyl ester methyl glycoside (**1**) is compatible with a 2,6-pyranoside ring structure. The anomeric configuration of the methyl glycoside group was assumed to be α and in axial orientation in order to accommodate the bulky methoxycarbonyl group in an equatorial orientation. The axial orientation of H-5 and -6 was deduced from the large coupling constants with H-4

TABLE I

AUTOCATALYTIC HYDROLYSIS[a] OF COMPOUND **4**

Reaction time (h)	*Products (%)*	
	Reducing compounds	*Inorganic phosphate*
12	56	10.5
36	70	16.3
70	100	29

[a]A 0.1M solution of **4** was heated at 70° for the time indicated. Aliquots were diluted 100-fold for assay of reducing compounds[14]. A standard curve was obtained with a sample of **5** judged to be pure by 3-dehydroquinate synthase assay. Inorganic phosphate was determined by the procedure of Ames and Dubin[15] on the same diluted aliquots.

and -5, respectively, and is in accord with the *arabino* configuration of the compound. The large coupling constant of H-3*a* with H-4 is in accord with the axial orientation of H-4. Surprisingly, the signal of H-5 was shifted upfield relative to that of H-6, contrary to the expected shift upfield of the signal of H-6, such as observed for the octulosonic acids[11]. The ^{1}H-n.m.r. spectrum of **5** was quite similar to that of **1**. However, the signal for H-6 in **5** occurred at a lower field and closer to the bulk of the signals of H-4 and -7. A common feature of ^{1}H-n.m.r. spectra of both compounds **1** and **5** is the separation of the signals of H-7 and -7′ into two distinct signals, as found also for octulosonic acid derivatives[11]. A signal of low intensity at δ 3.04 due to a methoxyl group was observed owing to a small proportion of **4**.

EXPERIMENTAL

General methods. — ^{1}H-N.m.r. spectra were recorded with Bruker 250 or 300 MHz instruments, and i.r. spectra with a Perkin–Elmer infrared spectrophotometer model 21. Elemental and methoxyl group analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN 37821.

Methyl (methyl 3-deoxy-D-arabino-*2-heptulopyranosid)onate* (**1**). — This compound was prepared as described previously[6]; ^{1}H-n.m.r. (250 MHz, ^2H_2O): δ 1.54 (dd, 1 H, $J_{3a,3e}$ 12.5, $J_{3a,4}$ 12 Hz, H-3*a*), 2.15 (dd, 1 H, $J_{3e,3a}$ 12.5, $J_{3e,4}$ 5.5 Hz, H-3*e*), 3.04 (s, 3 H, CH_3O), 3.21 (t, 1 H, $J_{5,4}$ 10.5, $J_{5,6}$ 10.5 Hz, H-5), 3.38 (ddd, 1 H, $J_{6,5}$ 10.5, $J_{6,7}$ 5.5 Hz, H-6), 3.59 (d, 1 H, $J_{7,6}$ 5.5 Hz, H-7), 3.66 (s, 3 H, CO_2CH_3), 3.7 (dd, 1 H, $J_{7',6}$ 2.5, $J_{7',7}$ 12.5 Hz, H-7′), and 3.75 (m, 1 H, H-4).

[*Methyl (methyl 3-deoxy*-D-arabino-*2-heptulopyranosid)onate*] *diphenyl 7-phosphate* (**2**). — A solution of diphenylphosphoric chloride (5.37 g, 20 mmol) in dry benzene (30 mL) was added slowly (1 h) with stirring to a solution of **1** (4.72 g, 20 mmol) in dry pyridine (40 mL) at −10° under exclusion of moisture. The solution was stirred for an additional h at 5° and kept overnight at 5°. After being kept

for 4 h at room temperature it was evaporated *in vacuo*, the residue was dissolved in chloroform (150 mL), and the solution washed twice with 5% sulfuric acid (saturated with sodium sulfate), water, 5% sodium hydrogencarbonate, and water. The dried chloroform solution was concentrated *in vacuo* to a yellow syrup which was dissolved in anhydrous methanol (100 mL), and the solution boiled under reflux for 5 min with a small amount of charcoal. The colorless filtrate was concentrated *in vacuo* to dryness (yield 7.9 g, 84%); syrup, ν_{max}^{KBr} 2.92, 3.25 (CH arom.), 3.40, 3.52, 5.70 (CO_2CH_3), 6.27, 6.71, 6.83 (arom.), 6.95, 8.43 (P–O–C arom.), 8.61 (P=O, H bonded), and 10.44 μm (P–O–C aliphatic).

*Methyl (methyl-3-deoxy-*D-arabino-*2-heptulopyranosid)onate dihydrogen 7-phosphate* (**3**). — A solution of **2** (7.9 g, 16.9 mmol) in anhydrous ethanol (100 mL) was treated with Adams' catalyst (0.5 g), and the suspension was stirred with hydrogen[12] (generated in a separate vessel). After 3 h, fresh catalyst (0.5 g) was added and stirring under hydrogen continued until the theoretical amount had been taken up (1 h; consumed: 37 mL of M sodium borohydride; calc 36 mL). The catalyst was removed and the filtrate concentrated *in vacuo* to dryness (yield 5.4 g, quantitative); syrup; the i.r. spectrum (KBr) showed no absorption for aromatic bands.

*(Methyl 3-deoxy-*D-arabino-*2-heptulopyranosid)onic acid dihydrogen 7-phosphate* (**4**) — A solution of **3** (3.16 g, 10 mmol) in 0.2M sodium hydroxide in 50% aqueous methanol (225 mL) was stirred for 4 h at room temperature. Moist Dowex 50 (H^+) cation-exchange resin (50 mL) was added, and stirring continued for 15 min. The resin was removed by filtration, and the combined filtrate and washings were concentrated *in vacuo* to dryness (yield 3 g, quantitative); syrup.

The tris(cyclohexyl)ammonium salt was prepared by treating a solution of **4** (175 mg, 58 mmol) in water (5 mL) with freshly distilled cyclohexylamine to pH 9, and concentrating *in vacuo* to dryness (yield 313 mg, 90%); white crystals. An analytical sample was prepared by dissolving the crystals (130 mg) in hot methanol and adding acetone to slight opalescence (yield 90 mg); m.p. 155–158° (dried *in vacuo* at room temperature).

Anal. Calc. for $C_{26}H_{54}N_3O_{10}P \cdot 3\ H_2O$: C, 47.77; H, 9.19. Found: C, 47.92; H, 9.23.

Further drying of the hydrated salt for 2 h at 100° *in vacuo* (2 mm Hg) gave the anhydrous salt.

Anal. Calc. for $C_{26}H_{54}N_3O_{10}P$: C, 52.07; H, 9.06; N, 7.00; P, 5.17. Found: C, 51.81; H, 9.18; N, 6.84; P, 5.99.

*3-Deoxy-*D-arabino-*2-heptulosonic acid 7-phosphate* (**5**). — A solution of **4** (5 g, 16.5 mmol) in 20mM hydrochloric acid (500 mL) was boiled under reflux for 2 h, and the pH of the resulting yellow solution was adjusted to 6 with M ammonium hydroxide. The volume was decreased *in vacuo* to ~50 mL, the pH adjusted to 7, and 2M barium bromide (75 mL) added. An amorphous precipitate of barium phosphate was removed by centrifugation and absolute ethanol (300 mL) added to the combined supernatant solution and washings (150 mL). The solid was collected by

centrifugation, washed twice with cold 70% ethanol and once with absolute ethanol, and dried *in vacuo* in the presence of phosphorus pentaoxide at room temperature (yield 4.1 g, 44% of barium salt tetrahydrate)[9].

The tripotassium salt, prepared[9] from the barium salt of **5**, was assayed with highly purified dehydroquinate synthase from *Escherichia coli*[3]. Only 85% of **5** was converted into 3-dehydroquinate. (In a control incubation with pure[9] **5**, the substrate disappeared completely.) The rate of consumption of **5** was monitored by the periodate–thiobarbiturate assay[13]. In this assay, **4** gave an E_{max} (72 000) essentially equal to that of **5** (68 000), as would be expected from the formation of 2,4-dioxobutyric acid from both compounds by periodate cleavage under acidic conditions. Therefore, the material unreactive in the enzyme-catalyzed conversion to 5-dehydroquinate (15%) is likely to have been **4** that escaped hydrolysis to **5**. This interpretation was in accord with the *O*-methyl group analysis of **5**, which showed the presence of methoxyl groups corresponding to 14% of **4**.

Anal. Calc. for $C_7H_9Ba_{1.5}O_9(OCH_3)P$ (**4**): CH_3O, 6.14. Found (**5**): 0.87.

The barium salt of **5** (purity 85%), isolated as just described was further purified as follows. A suspension of the salt (563 mg; 1 mmol) in water was de-ionized with Dowex 50 (H^+) cation-exchange resin. The pH of the combined filtrate and washings was brought to 8 with 2M ammonium hydroxide, and the solution (adjusted to 250 mL) was passed through a column (1.5 × 15.5 cm) of Dowex 1-X8 (Cl^-, 200–400 mesh). The column was washed with water (150 mL), 10mM hydrochloric acid (600 mL), 20mM hydrochloric acid (150 mL), and water (150 mL). Elution with 0.25M ammonium bromide gave **5** in fractions 2 and 3 (20 mL each) as assayed by the periodate–thiobarbiturate method. The solution was concentrated to a small volume and 2M barium bromide (3 mL) was added. The barium salt was isolated as described earlier (yield 270 mg, 46%), white solid; biological activity, 91%.

Alternatively, a solution of **4** (2.5 g) in 10mM hydrochloric acid (300 mL) was boiled under reflux for 1 h and the barium salt isolated as described earlier (yield 1.7 g, 40%); purity by enzymic assay, 70%.

Anal.: CH_3O, 2.01 (corresponds to 31% of **4**).

The product (1.7 g) was de-ionized, the acid was boiled under reflux with 20mM hydrochloric acid (150 mL) for 2 h, and the barium salt re-isolated (yield 1.2 g, 70%).

Anal.: Found by enzymic assay: 95%; CH_3O, 0.317 (5.2% of **4**).

^{1}H-N.m.r. (300 MHz, ^2H_2O; tripotassium salt): δ 1.7 (t, 1 H, $J_{3a,3e}$ 13.4, $J_{3a,4}$ 13.4 Hz, H-3*a*), 2.04 (dd, 1 H, $J_{3e,3a}$ 13.4, $J_{3e,4}$ 5.3 Hz, H-3*e*), 3.42 (t, 1 H, $J_{5,4}$ 10, $J_{5,6}$ 10 Hz, H-5), 3.69 (m, 1 H, H-6), 3.80 (m, 1 H, H-7), 3.88 (m, 1 H, H-7′), and 3.96 (m, 1 H, H-4).

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