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THE PREPARATION OF METHYL 5-DEOXY-5-(DIHYDROXYPHOSPHINOYL)HYDROXYMETHYL-2,3-*O*-ISOPROPYLIDENE-β-D-RIBOFURANOSIDE, A PRECURSOR TO A HYDROXYMETHYLENE ANALOG OF D-RIBOSE 5-PHOSPHATE*

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ABSTRACT

The synthesis of a precursor of 5-deoxy-5-(dihydroxyphosphino)hydroxymethyl-D-ribose, an isosteric analog of D-ribose 5-phosphate, is described. This compound differs from the natural phosphate by the incorporation of a phosphonic acid residue in place of the normal phosphate monoester group, and by the introduction of a hydroxyl group at the C atom linking the phosphorus atom to the carbohydrate portion of the molecule. The purpose of this hydroxyl group was to provide, to the phosphonic acid residue, a hydrophilic character that is normally lacking in simple phosphonic acid analogs of natural phosphates.

INTRODUCTION

Phosphonic acids as nominally isosteric analogs of natural phosphate metabolites have received increased interest in the past few years[2,3]. The present report describes the synthesis of a phosphonic acid analog of D-ribose 5-phosphate (**1**). The isosteric methylene analog **2**, related to **1**, has been reported previously by Moffatt and Jones[4,5]. We report now the preparation of the hydroxymethylene compound **9**, a precursor to the hydroxymethylene analog **3** related to 5-deoxy-5-(phosphono)hydroxymethyl-D-ribose.

Although the simple methylene analogs are often able to substitute quite well for the natural phosphates in biochemical processes[2], it has been noted for several enzyme systems that such analogs may not be recognized. Two examples of this are: (a) the failure of (*S*)-3,4-dihydroxybutyl-1-phosphonic acid to participate in reactions mediated by acyl coenzyme A: *sn*-glycerol-3-phosphate acyltransferase[6],

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and (b) the failure of methylenediphosphonic acid to serve as an inhibitor of a variety of pyrophosphatases[7,8].

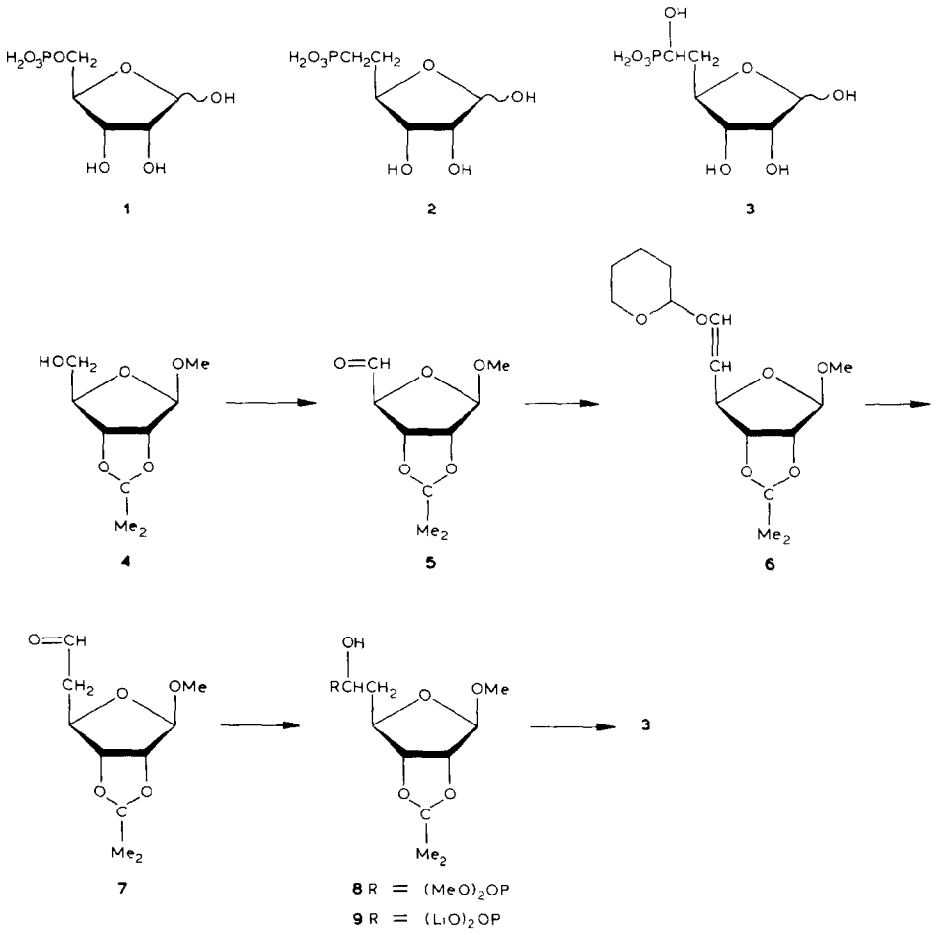
For both of these systems, the difficulties appear to be overcome by the incorporation of a hydroxyl group at position 2 from the phosphorus atom[7-9], or by introducing a phosphoramide linkage in place of the normal ester linkage[10,11]. In either situation, it is presumed that the introduced function, with its unshared electron pair, is capable of replacing the binding capability of the normal ester oxygen atom, which a methylene group is not. The present synthesis was directed toward the preparation of an analog in the carbohydrate series, wherein such an α-hydroxyl group is present.

RESULTS AND DISCUSSION

Several approaches to the synthesis of **3** were considered. One involves the generation of a substituted vinylphosphonate diester and its subsequent hydration. A second approach incorporates, at an early stage, the proper number of carbon atoms and functionality with an aldehyde function at the position ultimately to bear the phosphorus and hydroxyl groups. Then, phosphonylation would be accomplished by reaction either with a dialkyl phosphite in the presence of a base, or by reaction with a trisilylphosphite reagent, as described by Sekine *et al.*[12].

A major difficulty was encountered in attempting the synthesis through the vinylphosphonate diester. The substituted vinylphosphonate diester could not be generated from the aldehyde cleanly, or in good yield. As previously described[13], attempts to perform the reaction of an aldehyde bearing another acetal function with a phosphonate anion reagent, in an ether or dipolar aprotic solvent, resulted in a multitude of side-reactions involving the acetal linkage, the solvent system (hexane or another nonpolar, aprotic medium) used in that work[13] was also of no utility here due to the low solubility of the carbohydrate derivative.

The second approach was successful. Methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside (**4**) was oxidized with a pyridine complex of chromic anhydride in dichloromethane, as previously reported[14], to generate the aldehyde **5**. The enol ether **6** was then formed by reaction of **5** with the ylide produced upon treatment of triphenyl (oxan-2-yloxymethyl)phosphonium chloride with a base[15]. Several attempts at acid-catalyzed cleavage of the enol ether were made; the best conditions that left the remaining acetal function intact involved warming for several hours with 80% aqueous acetic acid. The resultant aldehyde **7**, homologous with **5** and having the proper number of carbon atoms for the target molecule, was then ready for phosphorus incorporation. This was accomplished by a modified Abramov procedure with dimethyl phosphite in benzene and addition of sodium methoxide for anion generation. The resultant adduct phosphonate diester **8** was transformed into the dilithium salt **9** by treatment with bromotrimethylsilane to generate the bis(trimethylsilyl) ester, followed by facile hydrolysis to give the free acid on water



addition. Finally, treatment with lithium hydroxide afforded **9**, which is the most convenient form for storage of **3**.

It is noteworthy that, in the ylide reaction of **5** to generate **6**, only a single spot could be detected for the product under all chromatographic conditions used. In spite of this, the ^{1}H-n.m.r. spectrum indicated the presence of a pair of isomers differing in geometry about the olefinic linkage. The two components, shown to be present in approximately equal amounts by integration, were distinguished by four signals, each a doublet: a downfield pair with J values of 13 and 6 Hz, and a corresponding upfield pair. Thus, an approximately equal distribution of *E*- and *Z*-isomers is indicated. Moreover, the signals involved are relatively broad, presumably the result of a mixture of diastereoisomers being present in each case, which suggests a stereochemical differentiation at C-2 of the oxanyl ring. As this geometrical differentiation is destroyed in the subsequent reaction, no further effort at

separation was made. A second point of stereochemistry concerns the preparation of **8**. A new chiral center was generated upon reaction of dimethyl phosphite with the prochiral aldehyde carbon of **7**, and one would anticipate the formation of a pair of diastereoisomers. However, no positive evidence for the presence of two diastereoisomers was found in the ^{1}H-n.m.r. spectrum, nor could any separation of materials be observed under any chromatographic conditions used. Neither could any stereochemical differentiation be made for the dilithium salt **9**.

EXPERIMENTAL

General. — All chemicals were of reagent grade and were used without further purification with the following exceptions: chloroform and dichloromethane were distilled in the presence of phosphorus pentaoxide, pyridine was dried in the presence of calcium hydride and distilled, oxolane was distilled in the presence of lithium aluminum hydride, and methanol was redistilled. Thin-layer chromatography was performed on Brinkman SIL N-HR precoated sheet. Silica gel for preparative chromatography was obtained from Baker (60-200 mesh). I.r. spectra were recorded using a Perkin–Elmer 598 spectrophotometer, and ^{1}H-n.m.r. spectra with a Varian EM-360 spectrometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TE 37921.

*Methyl 5-deoxy-2,3-O-isopropylidene-6-O- (oxan-2-yl)-β-*D*-ribo-hex-5-eno-furanoside* (**6**). — To a suspension of triphenyl-(oxan-2-yloxymethyl)phosphonium chloride (9.1 g, 21 mmol) in oxolane (40 mL), cooled to −10°, was added, dropwise, a 1.6M solution of 1-butyllithium in hexane (15 mL, 22 mmol). The solution was stirred under nitrogen for 0.5 h until the salt dissolved and a deep-red color developed. A solution of methyl 2,3-*O*-isopropylidene-β-D-*ribo*-pentodialdo-1,4-furanoside[14] (**5**) (4.04 g, 20 mmol) in oxolane (10 mL) was added dropwise. The solution was allowed to warm to 0° for 1 h, and then was kept for 20 h at room temperature. The solvent was evaporated under reduced pressure, and the residue dissolved in chloroform (100 mL) and washed with aqueous ammonium chloride (3 × 10 mL). After being dried (magnesium sulfate), the solution was evaporated, and the residue extracted with ether (5 × 20 mL). The ether extracts upon evaporation yielded a dark-yellow oil. It was purified by column chromatography in a column (4 × 50 cm) of silica gel (200 g), which was eluted with 4:1 (v/v) dichloromethane–methanol. Compound **6** was eluted in the 560–820-mL fraction as an oil (3.9 g, 65% yield), $[\alpha]_D^{27}$ −15.2° (*c* 0.15, ethanol); t.l.c. (4:1, v/v dichloromethane–methanol) R_F 0.82; $\nu_{max}^{CHCl_3}$ 1665 and 1630 cm^{-1} (olefinic linkage); ^{1}H-n.m.r. (chloroform): δ 6.57 (d, 0.5 H, *J* 13 Hz, *HC*=CC *trans*), 6.30 (d, 0.5 H, *J* 6 Hz, *HC*=CC *cis*), 5.32 (t, 1 H *J* 14 Hz, *HC*OCH=), 4.63 (d, 0.5 H, *J* 6 Hz, *HC*=CO *cis*), 4.55 (d, 0.5 H, *J* 13 Hz, *HC*=CO *trans*), 5.00–4.30 (m, 4 H, D-ribose ring H), 3.90–3.70 (m, 2 H, CH_2O), 3.45 (s, 3 H, OCH_3), 1.95–1.70 (m, 6 H, $CH_2CH_2CH_2$ of oxanyl ring), 1.62 (s, 3 H, C–CH_3), and 1.35 (s, 3 H, C-CH_3).

Anal. Calc. for $C_{15}H_{24}O_6$: C, 59.98; H, 8.05. Found: C, 60.16; H, 8.20.

*Methyl 5-deoxy-2,3-O-isopropylidene-β-*D-ribo-*hexodialdo-1,4-furanoside* (**7**). — To **6** (5.75 g, 19.2 mmol) was added 80% aqueous acetic acid (30 mL), and the reaction mixture stirred for 5 h with heating to 45–50° until no trace of the starting material remained as could be detected by t.l.c. Acetic acid was evaporated under reduced pressure, and the residue made neutral with aqueous sodium hydrogencarbonate and extracted with chloroform (5 × 20 mL). The extracts were combined, washed with water (2 × 5 mL), dried (magnesium sulfate), and evaporated to give a crude product mixture that exhibited three spots on t.l.c. (R_F 0.66, 0.47, and 0.33; 4:1, v/v, dichloromethane–acetonitrile). The mixture was chromatographed in a column (4 × 50 cm) of silica gel (200 g), eluted with 4:1 (v/v) dichloromethane–acetonitrile, the desired product being eluted in the 315–495-mL fraction. The aldehyde **7** (2.9 g, 70% yield) was isolated as an oil, $[\alpha]_D^{27}$ −64.6° (*c* 0.12, ethanol); t.l.c. (4:1, v/v, dichloromethane–acetonitrile) R_F 0.65; $\nu_{max}^{CHCl_3}$ 1725 cm^{-1} (carbonyl); ^{1}H-n.m.r. (chloroform): δ 9.72 (t, 1 H, *J* 2 Hz, *H*CO), 5.00–4.40 (m, 4 H, D-ribose ring H), 3.34 (s, 3 H, OCH_3), 2.66 (dd, 2 H, *J* 2, 8 Hz, CH_2), 1.40 (s, 3 H, C–C–CH_3), and 1.23 (s, 3 H, C–CH_3).

Anal. Calc. for $C_{10}H_{16}O_5$: C, 55.54; H, 7.45. Found: C, 55.78; H, 7.52.

*Methyl 2,3-O-isopropylidene-5-deoxy-5-(dimethoxyphosphinoyl)hydroxymethyl-β-*D-*ribofuranoside* (**8**). — To a solution of aldehyde **7** (2.8 g, 13 mmol) and dimethyl phosphite (1.65 g, 15 mmol) in benzene (10 mL) was added a 25% solution of sodium methoxide in methanol (5 drops), at which time an exothermic reaction began. The reaction was stirred for 3 h at 40°, and an additional 12 h at room temperature. Chloroform (100 mL) was added, and the solution washed with saturated, aqueous ammonium chloride until neutral and dried (sodium sulfate). The solvent was evaporated under reduced pressure to give a crude product (4 g) that was purified by chromatography in a column (4 × 50 cm) of silica gel (200 g), which was eluted with 3:1 (v/v) ethyl acetate–dichloromethane. Compound **8** was eluted in the 1500–2250-mL fraction as an oil (3.0 g, 71% yield), $[\alpha]_D^{27}$ −26.4° (*c* 1.6, ethanol); t.l.c. (3:1, v/v, ethyl acetate–dichloromethane) R_F 0.31; $\nu_{max}^{CHCl_3}$ 3570, 3300, 1240, and 1210 cm^{-1}; ^{1}H-n.m.r. (chloroform): δ 5.05–4.10 (m, 5 H, D-ribose ring and C*H*OH), 3.90 (d, 6 H, *J* 12 Hz, CH_3OP), 3.44 (s, 3 H, CH_3OCH), 3.42 (s, 1 H, O*H*), 2.10 (m, 2 H, CHCH_2CH), 1.50 (s, 3 H, C–CH_3), and 1.35 (s, 3 H, C–CH_3).

Anal. Calc. for $C_{12}H_{23}O_8P$: C, 44.17; H, 7.10, P, 9.49. Found: C, 44.27; H, 6.70; P, 9.85.

*Methyl 2,3-O-isopropylidene-5-deoxy-5-(dilithiumphosphono)hydroxymethyl-β-*D-*ribofuranoside* (**9**). — To a solution of **8** (2.7 g, 8.3 mmol) and triethylamine (0.84 g, 8.3 mmol) in tetrachloromethane (15 mL) was added dropwise bromotrimethylsilane (6.2 g, 41 mmol) under a nitrogen atmosphere at 0–5°. The mixture was allowed to warm to room temperature and was stirred for 12 h. The precipitate was filtered and washed with tetrachloromethane. The combined tetrachloromethane solutions were evaporated under reduced pressure. To the residue were added 1,4-dioxane (10 mL) and water (10 mL), and the mixture was stirred

for 12 h at room temperature. The solvent was evaporated, and the oily residue dissolved in ethanol (10 mL) and made neutral with a 2.5% solution of lithium hydroxide in 3:1 (v/v) ethanol–water. The solution was evaporated under reduced pressure to give a solid material that was washed with acetone and ether to yield **9** as the monohydrate (2.10 g, 77% yield), $[\alpha]_D^{27}$ −39.5° (*c* 3.3, water); t.l.c. (9:1, v/v, methanol–water), R_F 0.56, ^{1}H-n.m.r. (D_2O): δ 4.95 (m, 1 H, C*H*OH), 4.35–3.45 (m, 4 H, ribose ring H), 3.30 (s, 3 H, OCH_3), 1.85 (m, 2 H, PCHCH_2), 1.45 (s, 3 H, C–CH_3), and 1.35 (s, 3 H, C–CH_3).

Anal. Calc. for $C_{10}H_{17}Li_3O_8P \cdot H_2O$: C, 36.60; H, 5.83; P, 9.44. Found: C, 36.60; H, 5.78; P, 9.61.

The deprotected free acid **3** was generated by dissolution of **9** in a 25-fold amount of water and stirring with Dowex 50 (H^+, 10 equiv.). The resin was removed by filtration and the solvent partially evaporated under reduced pressure to remove extraneous organic compounds.

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