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ISOSTERES OF NATURAL PHOSPHATES. 8. THE PREPARATION AND BIOLOGICAL PROPERTIES OF 1-DEOXY-1-DIHYDROXYPHOSPHINYLMETHYLFRUCTOSE, AN ANALOGUE OF FRUCTOSE-1-PHOSPHATE.[1]

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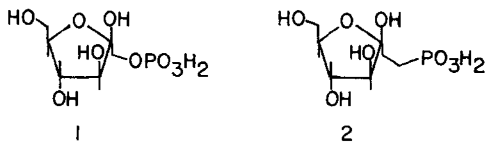
Numerous phosphonic acids have been synthesized and tested for use as analogues of natural phosphates in biological systems.[2] Of particular interest are those analogues which are capable not only of involvement in the same enzymatic processes as the natural materials, but also of entering intact cells. In regard to this latter point, on the basis of prior experience, one may predict that phosphonic acids will be transported into intact cells if they are isosteric with natural phosphates for which transport systems are present or can be induced. Thus the hexose phosphate transport system[3] of Escherichia coli would appear to provide a reasonable route for administration of a metabolic regulator in the form of a phosphonic acid isosteric with one of several natural carbohydrate phosphates.

The phosphonic acdi analogue of fructose-1-phosphate (1), 1-deoxy-1-dihydroxyphosphinylmethylfructose (2), would be considered on this basis to be a likely candidate as an in vivo regulator for bacterial growth. In fact, this prediction has now been realized with the synthesis and testing of (2). We find that 1-deoxy-1-dihydroxyphosphinylmethylfructose, at a concentration of 2 mM, inhibits the growth of strains of Escherichia coli which are constitutive for the hexose phosphate transport system.

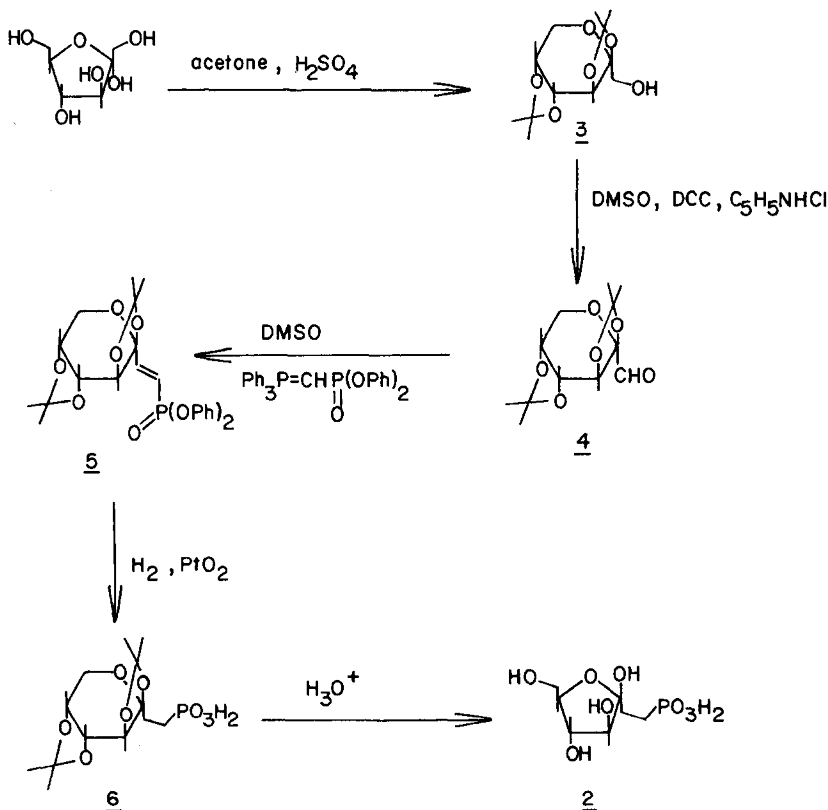


We herein describe the synthesis (outlined in Scheme 1) of this isostere of fructose-1-phosphate and briefly note the biological data. It is expected that this analogue will find

significant value both as an in vivo regulator and as a probe of biochemical mechanisms.

Materials

The 2,3:4,5-di-O-isopropylidene-β-D-fructopyranose (3) and 2,3:4,5-di-O-isopropylidene-β-D-arabino-hexosulo-2,6-pyranose (4) were prepared according to the methods of Brady,[4] and Lowe, et al.,[5] and the diphenyl triphenylphosphoranylidenemethylphosphonate by the method of Jones, et al.[6] All other chemicals were from commercial sources and used without further purification with the following exceptions: chloroform was distilled over phosphorus pentoxide, and dimethyl sulfoxide was vacuum distilled over calcium hydride. Silicic acid for chromatography was from Sigma (325 mesh). Infrared spectra were measured using a Perkin-Elmer 237-B spectrophotometer and NMR spectra were measured using a Varian EM-360 instrument. All elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee, and were within acceptable limits.



Preparation of 1,2-dianhydro-1,2-dideoxy-3,4:5,6-di-O-isopropylidene-β-D-fructo-heptulopyranose 1-diphenyl-phosphonate (5).

Diphenyl triphenylphosphoranylidenemethylphosphonate (1.500 g, 3.05 mmol) was added to 2,3:4,5-di-O-isopropylidene-β-D-arabino-hexosulo-2,6-pyranose (0.710 g, 3.03 mmol) in 15 ml of dry dimethyl sulfoxide and the reaction mixture was stirred at ambient temperature for 2 days. After the solvent was removed under reduced pressure, the residue was dissolved in 2 ml of chloroform and passed through a column of silicic acid (20 g). The desired product was eluted from the column along with triphenylphosphine oxide by-product using 400 ml of chloroform. Upon evaporation of the solvent under reduced pressure low boiling petroleum ether was added to precipitate the triphenylphosphine oxide which was removed by filtration. The solvent was then removed under reduced pressure to yield 0.710 g (48%) of pure 5 as a syrup which exhibited a single spot of R_f=0.65 on thin layer chromatography (Eastman Chromagram Sheet, Silica Gel) developed with 10:90 methanol:chloroform and visualized either with iodine or molybdate spray reagent. Analysis, $C_{25}H_{29}O_8P$ requires C 61.47%, H 5.98%; found: C 61.60%, H 6.27%. NMR ($CDCl_3$) 1.24δ,3H(s); 1.31δ, 3H(s); 1.47δ,3H(s); 1.50δ,3H(s); 3.55-4.66δ,5H(m); 6.03-6.90δ,2H(m); 7.15δ,10H(broad).

Preparation of 1,2-dideoxy-3,4:5,6-di-O-isopropylidene-β-D-fructo-heptulopyranose 1-phosphonic acid (6).

In 15 ml of absolute ethanol was placed 240 mg (0.49 mmol) of 5 with 80 mg of PtO_2 and subjected to hydrogenation-hydrogenolysis at 1 atm hydrogen pressure for 48 hr. The catalyst was removed by filtration through Celite and the solvent was evaporated at reduced pressure. The residual semisolid 6 (140 mg, 84%) was hydroscopic and not ameanable to elemental analysis. It however exhibited spectra in accord with the postulated structure, the absence of aromatic proton signals in the NMR taken as an indication of completion of reaction, and was used without further purification. NMR ($CDCl_3$) 1.24δ,3H(s); 1.38δ,3H(s); 1.42δ,3H(s); 1.56δ,3H(s); 2.07δ,4H(broad); 3.53-4.70δ,5H(m).

Preparation of 1-deoxy-1-dihydroxyphosphinylmethylfructose (2).

The diacetonide 6 (140 mg, 0.41 mmol) was dissolved in 50 ml of water and there was added 3 g of Dowex 50 ion exchange resin which had been put in the acid form. This mixture was heated at 100° for 1 hr at which time the resin was removed by filtration and the water evaporated under

reduced pressure to yield 95 mg (89%) of pure 2 as a solid of mp 101-103°. The material exhibited a single spot of R_f=0.29 on paper chromatography (Whatman No. 3) developed with 60:10:30 n-propanol: water:conc. ammonia and visualized with molybdate spray reagent. The material analyzed as the hemihydrate; this water is tightly bound and could not be removed by leaving under vacuum (0.1 Torr) overnight at ambient temperature. Analysis, $C_7H_{15}O_8P$-1/2H_2O requires C 31.46%, H 6.04%; found: C 31.49%, H 6.21%. NMR (D_2O) 1.71-2.49δ,4H(m); 3.55-4.44δ,5H(m).

Biological Data

The inhibition of growth of two mutant strains of *E. coli* in the presence of 1-deoxy-1-dihydroxyphosphinylmethylfructose (2) was investigated. Both strains, RK 1042 and RK 1435,[7] are constitutive for the hexosephosphate transport system. The cultures were incubated at 37° and their growth was monitored turbidimetrically in a Klett colorimeter. At a 2 mM concentration of (2), both strains exhibited complete inhibition of growth. Further work on the details of the mode of inhibition is in progress.

Acknowledgement

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7. Both strains of *E. coli* were the generous gift of R. Kadner.