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Aldolase-Catalyzed Synthesis of Complex C_8 and C_9 Monosaccharides.[1]

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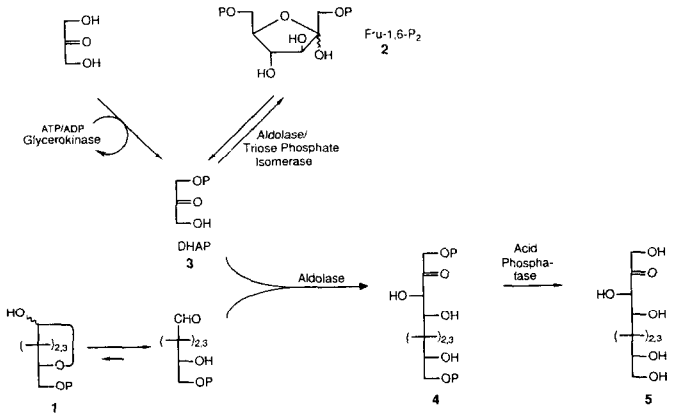
Summary. Fructose-1,6-diphosphate aldolase (E.C. 4.1.2.13) from rabbit muscle catalyzes the stereospecific aldol condensation of dihydroxyacetone phosphate with a variety of pentose-5-phosphates and hexose-6-phosphates. The aldol products can be dephosphorylated with acid phosphatase (E.C. 3.1.3.1) and the resulting free carbohydrates are peracetylated. These C_8 and C_9 sugars can serve as precursors to and analogs of KDO, sialic acids and related substances.

This letter demonstrates that rabbit muscle aldolase (E.C. 4.1.2.13, fructose-1,6-diphosphate aldolase) catalyzes the regiospecific aldol condensation of dihydroxyacetone phosphate (DHAP) with a number of C_5 and C_6 sugar monophosphates (eq 1[4]; Table I). This reaction (followed, if necessary by removal of phosphate



(1)

moieties from the product: **4**→**5**) provides a broadly applicable synthetic method that transfers stereochemistry present in hexoses and pentoses into C_8 and C_9 sugars and sugar phosphates. We are interested in these substances as precursors to and analogs of KDO, sialic acid and related sugars,[5] and as intermediates in complex synthesis.

Catalysis by aldolase is a useful method for preparing hexoses and pentoses using non-sugar aldehydes as substrates.[6-9] The C_3-C_4 bond is formed regiospecifically. Aldolase requires DHAP or a close structural analog as one substrate.[10] Although aldolase accepts a number of aldehydes as the second substrate, unphosphorylated hexoses and pentoses are accepted poorly or not at all. The central observation on which the present work is based is that hexose-6-phosphates and pentose-5-phosphates are good substrates for

rabbit muscle aldolase.[11] The phosphate groups in the products are helpful in isolations; they are easily removed with acid phosphatase (E.C. 3.1.3.1).

Table I summarizes starting materials, products, and yields. We have dephosphorylated, peracetylated and purified these compounds to homogeneity; stereochemical assignments are based on 500 MHz ^{1}H and 125 MHz ^{13}C NMR spectra of the peracetylated substances The following paragraphs outline experimental procedures.

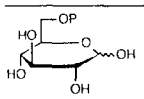
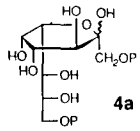
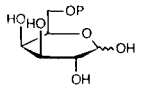
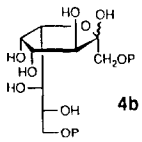
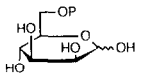
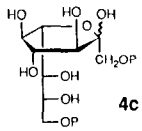
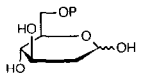
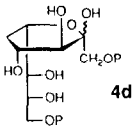
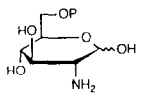
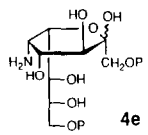
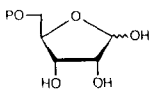
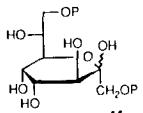
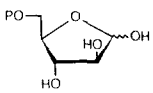
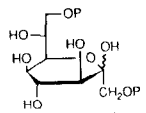
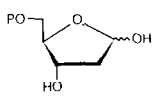
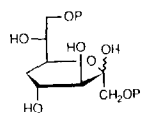
Method A: Fructose-1,6-diphosphate. Ribose-5-phosphate disodium salt dihydrate (1.55 g, 5 mmol) and fructose-1,6-diphosphate trisodium salt pentahydrate (1.25 g, 2.5 mmol) were dissolved in 20 mL of a 0.06 M diethanolamine-triethanolamine buffer. The pH was adjusted to 6.8 by the addition of 1 N HCl. Aldolase (100 U) and triosephosphate isomerase (500 U, E.C. 5.3.1.1) were added, and the solution was stirred at room temperature for 42 h, at which time enzymatic assay indicated 62% conversion. Barium acetate (2.6 g, 10 mmol) and acetone (50 mL) were added to the reaction mixture, and a white precipitate formed immediately. The reaction mixture was allowed to stand at 0 °C for 24 h, and the precipitate (the barium salt of compound **4f**) was collected (3.2 g).

Method B: Dihydroxyacetone phosphate. Ribose-5-phosphate disodium salt dihydrate (170 mg, 0.55 mmol) and dihydroxyacetone phosphate lithium salt (150 mg, 0.88 mmol) were dissolved in 10 mL of water, and the pH was adjusted to 6.8 using 1 N sodium hydroxide. Aldolase (50 U) was added and the solution was stirred at room temperature for 48 h, at which time both enzymatic assay and ^{1}H NMR analysis indicated an 82% conversion. Barium acetate (380 mg, 1.5 mmol) and acetone (30 mL) were added to the reaction mixture, and the solution was cooled to 0 °C. After 4 h, the barium salt of compound **4f** was collected as a white precipitate (308 mg).

Phosphate Cleavage and Acetylation. Water (5 mL) and ion exchange resin (Dowex 50 W x 8 H^{+} form, 0.50 g) were added to 250 mg of the barium salt **4f** formed by either method A or method B. The solution was stirred until the white precipitate dissolved. The ion exchange resin was removed by filtration, and the pH of the aqueous layer was adjusted to 5.2 using 1 N HCl. To this solution was added 60 U of acid phosphatase, and the reaction mixture was stirred at room temperature. After 48 h, the volatiles were removed by concentration in vacuo. The dry residue was treated with 10 mL of a 10:1 mixture of pyridine and acetic anhydride at 0 °C. After 48 h, t.l.c. analysis indicated the reaction was complete. The volatiles were removed by concentration in vacuo and the residue was subjected to flash chromatography (SiO_2, 1:1 ethyl acetate/hexane) and gave a clear colorless oil. ^{1}H NMR analysis revealed the material to be a mixture of stereoisomers (32% overall yield based on ribose-5-phosphate by method A and 54% overall yield by method B). This mixture was carefully rechromatographed and yielded one pure anomer, which was assigned the stereochemistry given in **4f** on the basis of spectroscopic evidence (in its peracetylated form).[12]

We have not yet defined the full range of sugar phosphates accepted as substrates by aldolase, but we note that the examples in Table I include pentoses, hexoses, a 2-deoxypentose and 2-deoxyhexose, and 2-aminohexoses. This range of substrates, together with the broad substrate specificity characteristic of aldolase, suggests that this reaction has general applicability. It thus combines the generality, stereospecificity, and ease of operation required to be a widely useful addition to the methodology of sugar synthesis.

Table I. Summary of starting materials and aldol products obtained from the aldolase-catalyzed reactions using either DHAP or FDP as an in situ source of the ketophosphate.

Sugar Phosphate	Yield [%] FDP	Yield [%] DHAP	Product
 Glc-6-P **1a**	36	58	 **4a**
 Gal-6-P **1b**	30	48	 **4b**
 Man-6-P **1c**	45	62	 **4c**
 2-Deoxy-glc-6-P **1d**	40	53	 **4d**
 2-Deoxy-2-amino-glc-6-P **1e**	30	46	 **4e**
 Rib-5-P **1f**	61	82	 **4f**
 Ara-5-P **1g**	35	48	 **4g**
 2-Deoxy-rib-5-P **1h**	45	58	 **4h**

References

1. Supported by NIH Grant GM 30367.
2. American Cancer Society Postdoctoral Fellow, Grant No. PF-2762, 1986.
3. Deutsche Forschungsgemeinschaft Postdoctoral Fellow 1985-1986.
4. The bracketed carbons in eq 1 respresent a variety of functional groups and stereochemistries accepted in the aldolase reaction.
5. Schauer, R. Trends in Biochem. Sci. **1985**, 357. Unger, F. M. Adv. Carbohydr. Chem. Biochem. **1981**, 38, 323. Sharon, N. "Complex Carbohydrates: Their Chemistry, Biosynthesis and Functions"; Addison-Wesley: Reading, MA 1975. Kennedy, J. F.; White, C. A. "Bioactive Carbohydrates in Chemistry, Biochemistry and Biology"; Ellis Horwood Limited: West Sussex, England 1983.
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7) Wong, C.-H.; Whitesides, G. M. J. Org. Chem. **1983**, 48, 3199. Wong, C.-H.; Mazenod, F. P.; Whitesides, G. M. ibid. **1983**, 48, 3493.
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10) We generate DHAP either by phosphorylation of dihydroxyacetone (Crans, D. C.; Whitesides, G. M. J. Am. Chem. Soc. **1985**, 107, 7019) or from fructose-1,6-diphosphate by the combined action of aldolase and triosephosphate isomerase.[7]
11) Octoluses and their 1,8-diphosphates have been isolated from plants and synthesized independently using aldolase based methods: Jones, J. K. N.; Septon, H. H. Can. J. Chem. **1960**, 38, 753. Kapuscinski, M.; Franke, F. P.; Flanigan, J.; MacLeod, J. K.; Williams, J. F. Carbohydr. Res. **1985**, 140, 69. The activity of hexose-6-phosphates as substrates for aldolase has not been previously investigated.
12) The NMR, IR and mass spectral data are consistent for compounds **4a-4h.** For compound **4f:** ^{1}H NMR (500 MHz, $CDCl_3$) 5.73 (d, J = 3.8 Hz, 1 H) 5.36 (apparent t, J = 5.4 Hz, 1 H), 5.26 (d, d, J = 5.7, 3.8 Hz, 1 H), 5.26-5.20 (m, 1 H), 4.60 (d, J = 12 Hz, 1 h), 4.44 (apparent t, J = 5.9 Hz, 1 H), 4.27-4.15 (m, 3 H, includes 4.23 (d, J = 12 Hz, 1 H)), 2.058 (s, 3 H), 2.052 (s, 3 H), 2.041 (s, 3 H), 2.035 (s, 3 H), 2.030 (s, 3 H), 2.026 (s, 3 H), 2.003 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.41, 169.85, 169.70, 169.42, 169.37, 168.95, 168.86, 107.99, 78.38, 75.73, 69.68, 69.55, 61.71, 61.49, 21.15, 19.95, 19.90; IR ($CHCl_3$) 3070, 1750, 1515, 1420, 1370 cm^{-1}; MS (chemical ionization, isobutane), m/e 535 (m^+ + 1, 1.2%), 475 (m^+ + 1 - AcOH, 41.6%).

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