(AB q, J = 12.00 Hz, $\Delta\nu$ = 68.16 Hz, 2 H), 3.98-3.62 (m, 4 H), 3.37 (s, 6 H), 3.26 (m, 1 H), 2.30 (m, 2 H), 2.05 (s, 3 H), 1.60 (m, 2 H), 1.32 (d, J = 6.24 Hz, 3 H), 1.26 (d, J = 6.24 Hz, 3 H) ppm.

Preparation of Benzyl 2-*O*-[α-Ethxoy-α-(trifluoromethyl)phenylacetyl]-β-L-oleandroside (22). Benzyl β-L-oleandroside (5 mg, 0.02 mmol) was dissolved in 2 mL of dry CCl_4, and 5 drops of dry pyridine were added, followed by distilled (-)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (0.09 g, 0.3 mmol). The solution was stirred 12 h at ambient temperature before being passed through a column of silica gel, eluting with 25% ethyl acetate in hexane, to yield 10 mg (100%) of the desired ester. GC and 360-MHz ^{1}H NMR analyses showed a 20:1 ratio of the desired diastereomeric esters. A 90-MHz ^{13}C NMR broad-band decoupled spectrum failed to show any diasteromeric material: IR (CCl_4) 3060, 3030, 2940, 1765, 1500, 1460, 1250, 1200, 1130, 1030, 925, 865 cm^{-1}; NMR ($CDCl_3$) 7.63 (m, 2 H), 7.39 (m, 8 H), 4.94 (t, J = 9.60 Hz, 1 H), 4.75 (AB q, J = 11.52 Hz, $\Delta\nu$ = 113.33 Hz, 2 H), 4.54 (dd, J_1 = 1.44 Hz, J_2 = 10.08 Hz, 1 H), 3.59 (d, J = 0.48 Hz, 3 H), 3.42 (m, 2 H), 3.33 (s, 3 H), 2.45 (ddd, J_1 = 1.44 Hz, J_2 = 4.80 Hz, J_3 = 12.48 Hz, 1 H), 1.69 (m, 1 H), 1.22 (d, J = 5.76 Hz, 3 H) ppm; $[\alpha]^{22}_D$ +39.25° (c 0.823). Anal. Calcd for $C_{24}H_{27}O_6F_3$: C, 61.53; H, 5.81; F, 12.17. Found: C, 61.32; H, 5.77; F, 12.30.

Acknowledgment. We appreciate the support of the National Institutes of Health (Grant GM-28056-03), the National Science Foundation (Grant CHE 8019770), and the partial suppot of the donors of the Petroleum Research Fund, administered by the American Chemical Society.

Registry No. α-1, 87037-59-0; β-1, 87037-60-3; **2** (isomer 1), 86971-99-5; **2** (isomer 2), 86972-00-1; **3**, 33106-64-8; **4**, 81445-44-5; **5**, 86972-01-2; **6**, 86972-02-3; **7**, 87037-61-4; **8**, 87037-62-5; **9**, 86972-03-4; **10**, 87037-63-6; **11**, 87037-64-7; **12**, 86972-04-5; **13**, 87037-65-8; **14**, 87037-66-9; **15**, 86972-05-6; **16**, 87037-67-0; **17**, 87037-68-1; **18**, 86972-06-7; **19**α, 86972-07-8; **19**β, 86972-08-9; **20**α, 86972-09-0; **20**β, 86972-10-3; **21**α, 86972-11-4; **21**β, 86972-12-5; **22**, 86972-13-6; **23**α, 86972-14-7; **23**β, 86972-15-8; oxalyl chloride, 79-37-8; 2(*R*)-(phenylmethoxy)propanol, 87037-69-2; 3-methoxypropene, 627-40-7; 4,5-dimethyl-2-fluoro-1,3-dioxa-2-boracyclopentane, 86972-16-9; (-)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, 39637-99-5.

Chemical and Enzymatic Syntheses of 6-Deoxyhexoses. Conversion to 2,5-Dimethyl-4-hydroxy-2,3-dihydrofuran-3-one (Furaneol) and Analogues[1]

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Received January 6, 1983

6-Deoxy-D-fructose 1-phosphate (6-deoxyF-1-P) forms when a solution containing D-fructose 1,6-diphosphate (FDP) and D-lactaldehyde is treated with the enzymes aldolase and triosephosphate isomerase (Scheme I). This transformation involves three reactions: aldolase-catalyzed cleavage of FDP to a mixture of dihydroxyacetone phosphate and D-glyceraldehyde phosphate, triosephosphate isomerase catalyzed equilibration of dihydroxyacetone phosphate and D-glyceraldehyde phosphate, and aldolase-catalyzed condensation of dihydroxyacetone phosphate and D-lactaldehyde to 6-deoxyF-1-P. An analogous process converts a mixture of FDP and L-lactaldehyde to 6-deoxysorbose 1-phosphate (6-deoxyS-1-P). Aldolase-catalyzed reaction of dihydroxyacetone phosphate, prepared separately, with D-lactaldehyde yields 6-deoxyF-1-P directly; similar reaction of dihydroxyacetone phosphate with α-hydroxybutyraldehyde yields a mixture of 6-methyl-6-deoxyhexose 1-phosphates. Acid-catalyzed hydrolysis of the sugar phosphates releases the corresponding free sugars. A mixture containing 6-deoxyhexoses is formed directly by base-catalyzed aldol condensation of dihydroxyacetone and D,L-lactaldehyde. Treatment of any of the 6-deoxyhexoses with acids generates 2,5-dimethyl-4-hydroxy-2,3-dihydrofuran-3-one (Furaneol, a flavor principle). Furaneol can also be prepared in moderate yields by hydrogenolysis of FDP and other hexose phosphates in alkaline media.

Introduction

This paper describes procedures using enzyme-catalyzed and conventional chemical steps for the preparation of unusual sugars. We have described previously the use of aldol condensations catalyzed by aldolase (EC 4.1.2.13, from rabbit muscle) as a route to isotopically labeled glucose 6-phosphate and fructose 6-phosphate.[3] The work reported here uses aldolase in analogous preparations of 6-deoxyhexoses and 6,7-dideoxyheptoses and compares enzymatic and conventional chemical routes to these substances. These deoxyhexoses are precursors to 2,5-dimethyl-4-hydroxy-2,3-dihydrofuran-3-one[4-6] (Furaneol, a caramel flavor component[2]). Because 6-deoxy sugars are relatively unusual in nature, we were interested in developing practical synthetic routes to them.

Rabbit muscle aldolase is commercially available and stable in the immobilized form. It requires dihydroxyacetone phosphate (DHAP)[7] as one reactant in the aldol

(1) Supported by the National Institutes of Health, Grants GM-26543 and GM 30367, and by Firmenich, SA. Inquiries should be addressed to G.M.W. at the Department of Chemistry, Harvard University, Cambridge, MA 02138.

(2) Firmenich, SA, Geneva, Switzerland. Furaneol is a registered trademark of Firmenich, Inc.

(3) Wong, C-H.; Whitesides, G. M. *J. Am. Chem. Soc.* **1983**, *105*, 5012. Attempted oxidation of D,L-glycerol phosphate (easily prepared from glycerol by reaction with $POCl_3$ in acetone in 50% yield) with bromine or hypochlorite resulted in low yields of product (~10% of the starting material was oxidized on treatment with these oxidants for 24 h at room temperature). The product contained both DHAP and D,L-glyceraldehyde 3-phosphate.

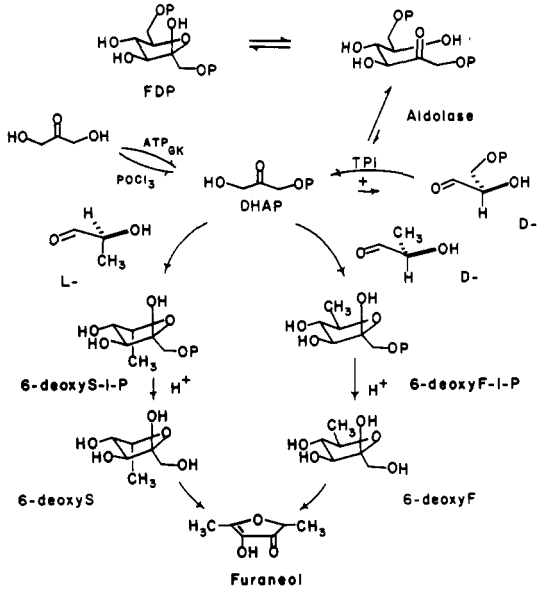
(4) Matsui, M.; Ogawa, T.; Tagaki, K. (T. Hagegawa, Co. Ltd.), Jpn. Kokai Tokkyo Koho 79, 19962, 15 feb 1979. In addition to 6-deoxyglucose, 6-deoxy-L-mannose has been converted to Furaneol. We have also found that both 6-deoxy-D-galactose (D-fucose) and 6-deoxy-L-galactose (L-fucose) are good starting materials for the preparation of Furaneol. Of the reaction conditions tested, piperidine acetate in absolute ethanol is the best acid-base catalyst system for the conversion and Furaneol has been prepared consistently in ~80% yield at ~80 °C from 6-deoxyhexoses with use of this catalyst.

(5) Hadyi, J. E. U.S. Patent 2 936 308.

(6) Prepared chemically from: 3-hexyne-2,5-diol (Re, L.; Maurer, B.; Ohloff, G. *Helv. Chim. Acta* **1973**, *56*, 1882-94); 2,5-dimethylfuran, and pyruvaldehyde (Büchi, G.; Demole, E. *J. Org. Chem.* **1973**, *38*, 123-5).

0022-3263/83/1948-3493$01.50/0

Scheme I. Preparation of 6-Deoxyhexoses and Furaneol

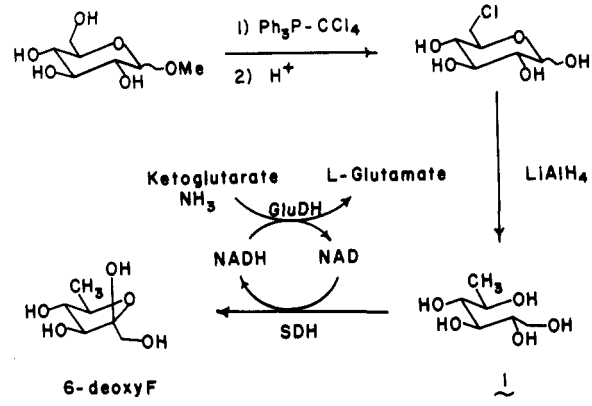


condensation but accepts, inter alia, a range of α-hydroxy aldehydes as the second.[3,8] The specific activity of aldolase as catalyst for the aldol condensation of DHAP with lactaldehyde or α-hydroxybutyraldehyde (~5 U/mg; 1 U = 1 μmol of product formed/min) is ~10% of that observed with use of its natural substrates, DHAP and D-glyceraldehyde 3-phosphate. Here we describe and compare enzyme-catalyzed procedures for the preparation of 6-deoxyhexose 1-phosphates from DHAP and α-hydroxyaldehydes and conventional chemical routes to phosphate-free 6-deoxyhexoses from dihydroxyacetone and α-hydroxy aldehydes. We also describe the conversion of these deoxy sugars to Furaneol and the preparation of this substance by reductive routes from hexose 6-phosphates.

Results

6-Deoxy-D-fructose (6-deoxyF) and 6-Deoxy-L-sorbose (6-deoxyS). Aldolase as Catalyst. Treatment of a mixture of FDP and D-lactaldehyde with coimmobilized aldolase and TPI yields 6-deoxyfructose 1-phosphate in ~80% yield; analogous reaction with L-lactaldehyde yields 6-deoxysorbose 1-phosphate (Scheme I). These treatments involve an initial aldolase-catalyzed reverse aldol cleavage of FDP to a mixture of DHAP and D-glyceraldehyde 3-phosphate. Equilibration of these substances is catalyzed by TPI; the equilibrium mixture contains 96% DHAP.[9] The DHAP then undergoes aldolase-catalyzed aldol condensation with the lactaldehyde and forms 6-deoxyhexose 1-phosphate. The equilibrium for the overall reaction favors condensation,[10] and the aldol reactions are stereospecific. Aldolase and TPI can be recovered from the reactions at their conclusion with high retention of their catalytic activity (78% and 75%, respectively, of their original activities) and reused. An alternate approach to these 6-deoxyhexose phosphates uses

Scheme II. Preparation of 6-Deoxy-D-fructose from Glucose (SDH, Sorbitol Dehydrogenase)



DHAP as starting material. DHAP can be prepared from dihydroxyacetone with use of either chemical or enzyme-catalyzed phosphorylation.[3] In this preparation, TPI is omitted. Both of these aldolase-catalyzed reactions generate products in high purity. These products are easily isolated in high yield.

To remove the phosphate moiety, solutions of the sugar phosphates were made acidic (pH ~1.0) with Dowex 50 (H^+) and maintained at 90 °C for 8 h. The inorganic phosphate released was removed by treatment with Dowex 1 (HCO_3^-). The free sugar was concentrated to an oily residue that appeared to be homogeneous by HPLC analysis.

From Glucose. Another route to 6-deoxyF starts with glucose (Scheme II). The most difficult step—the oxidation of 6-deoxy-D-glucitol (1) to 6-deoxyF—is catalyzed by sorbitol dehydrogenase (SDH, EC 1.1.1.14). In practice, this route is less convenient than that outlined in Scheme I because SDH is expensive. The reaction also requires nicotinamide cofactors, and it involves chemical steps that are sensitive to moisture.

Amberlite-Catalyzed Aldol Condensation. Amberlite IRA-400 (OH^-)[12] catalyzes the reaction of dihydroxyacetone and D,L-lactaldehyde and gives a mixture of products containing 6-deoxyF and 6-deoxyS (and their enantiomers) in ~80% yield as determined by HPLC analysis. This procedure is of no value as a sugar synthesis, but the mixture is useful for the preparation of Furaneol.

2,5-Dimethyl-4-hydroxy-2,3-dihydrofuran-3-one (Furaneol). Either 6-deoxyF or 6-deoxyS was converted into Furaneol at 80 °C in ~80% yield, using a mixture of piperidine and acetic acid (6:10 by weight) as catalyst and absolute ethanol as solvent. These conditions are similar to those reported previously for the conversion of 6-deoxy-D-glucose into Furaneol.[4] These conditions also converted the crude product mixture containing 6-deoxyhexoses obtained by Amberlite-catalyzed aldol condensation of dihydroxyacetone and lactaldehyde into Furaneol in 60% yield on the basis of dihydroxyacetone. This procedure seems the most practical route to Furaneol developed in this work but requires recrystallization or column chromatography if pure product is required.

Furaneol Analogues. Enzymatic procedures analogues to those described for the preparation of deoxyhexoses converted DHAP and D,L-α-hydroxybutyraldehyde to a

(7) Abbreviations: 6-deoxyF, 6-deoxy-D-fructose; 6-deoxyS, 6-deoxy-L-sorbose; G-deoxyF-1-P, 6-deoxy-D-fructose 1-phosphate; 6-deoxyS-1-P, 6-deoxy-L-sorbose 1-phosphate; F-1-P, D-fructose 1-phosphate; FDP, D-fructose 1,6-diphosphate; G-6-S, D-glucose 6-sulfate; HK, hexokinase; PFK, phosphofructokinase; TPI, triosephosphate isomerase.

(8) A crude preparation of aldolase from pea seeds accepts D,L-lactaldehyde as substrate: Hough, L.; Jones, J. K. N. *J. Chem. Soc.* **1952**, 4052–5.

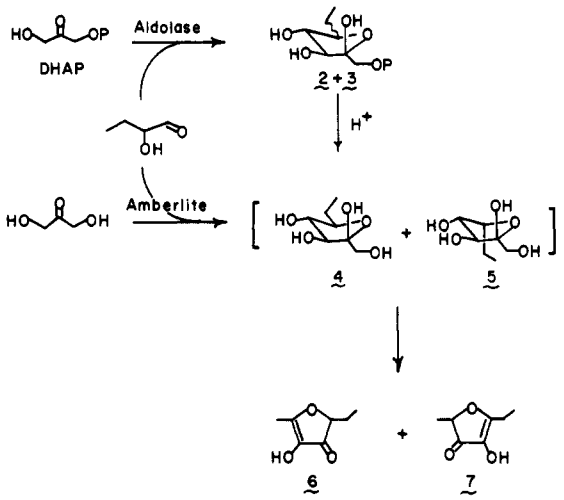
(9) Oesper, P.; Meyerhof, O. *Arch. Biochem. Biophys.* **1950**, *27*, 223–33.

(10) The equilibrium constant for FDP formation is 1.2×10^4 M^{-1}: Rutter, W. J. In "The Enzymes"; Boyer, P. D., Lardy, H. A., Hyrbäch, K., Eds.; Academic Press: New York, 1961; Vol. 5, p 341.

(11) The reagent is selective for primary alcohols: Whistler, R. Y.; Anisuzzaman, A. K. M. *Methods Carbohydr. Chem.* **1980**, *8*, 227–31. Slagle, J. D.; Huang, T. T. S.; Franzus, B. *J. Org. Chem.* **1981**, *46*, 3520–30.

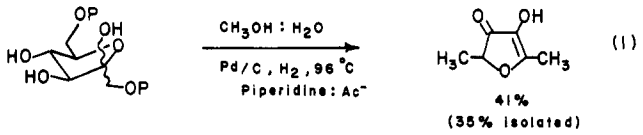
(12) The resin has been used as catalyst to prepare D,L-fructose in 78% yield from dihydroxyacetone and glyceraldehyde: Morgenlie, S. *Carbohydr. Res.* **1980**, *80*, 215–22.

Scheme III. Synthesis of Furaneol Analogues (the Amberlite-Catalyzed Reaction Generates a Diastereomeric Mixture)



mixture of products containing 6,7-dideoxy-D-heptulose 1-phosphate (2) and 6,7-dideoxy-L-heptulose 1-phosphate (3) in 78% yield. The related Amberlite-catalyzed aldol reaction yields 6,7-dideoxyheptoses in 70% yield (Scheme III). The mixture of 4 and 5, after removal of the phosphate moieties, was converted into Furaneol analogues (2-ethyl-4-hydroxy-5-methyl-2,3-dihydrofuran-3-one (6) and 5-ethyl-4-hydroxy-2-methyl-2,3-dihydrofuran-3-one (7)) in 50% yield; the corresponding yield from the Amberlite-catalyzed reaction was ~20% on the basis of dihydroxyacetone.

Furaneol by Hydrogenolysis of Hexose Phosphates. As an alternative route to 6-deoxyhexoses to be used in the synthesis of Furaneol, we explored the hydrogenolysis of several sugar phosphates. Although the detailed mechanism of these reactions remains unclear, under optimum conditions modest yields of Furaneol could be isolated directly from these reactions. Equation 1 gives



(1)

the best result obtained. Yields from other sugars are summarized in supplementary material to this paper.

Discussion

Schemes I and III illustrate the use of aldolase as a catalyst for the enantiospecific preparation of 6-deoxyhexoses and derivatives from simple precursors. These substances can be converted into Furaneol (and analogues) in good yield. Preparation of a mixture of 6-deoxyF-1-P and 6-deoxyS-1-P from FDP and D,L-lactaldehyde using a crude extract from pea seeds has been reported,[8] but the work did not demonstrate the stereochemistry of the aldol condensation. The procedures described in this paper using D- or L-α-hydroxy aldehydes as substrates illustrate enantiospecific aldol condensations catalyzed by rabbit aldolase. Amberlite ion-exchange resin (hydroxide form) is a good catalyst for *achiral* syntheses of mixtures of 6-deoxyhexoses; these mixtures can also be converted into Furaneol, but the yields are lower and the product purification more difficult than with use of the enzymatically prepared sugars. The yields on conversion of 6-deoxyhexose into Furaneol seem to be independent of the stereochemistry of the sugar.

The availability of DHAP determines the practicality of synthetic routes on the basis of aldolase. This substance is easily prepared from dihydroxyacetone, either by chemical or enzymatic phosphorylation;[3] it also can be generated in situ from FDP.

Experimental Section

Materials and Methods. Enzymes and biochemicals were obtained from Sigma. Pyruvaldehyde dimethyl acetal was from Aldrich. Other reagents were reagent grade. Phosphorus oxychloride was distilled before use. High-performance liquid chromatography (HPLC) was performed with a Waters μBondapak/carbohydrate column (0.4 × 30 cm) with aqueous acetonitrile (CH_3CN/H_2O = 85:15 v/v) as the mobile phase and differential refractometry for detection. UV spectra were taken with a Perkin-Elmer 552 spectrophotometer, equipped with a constant-temperature cell. ^1H (250 MHz) and ^{13}C (62.8 MHz) NMR spectra were obtained with a Bruker instrument. Enzyme immobilizations were carried out as described.[13] Sorbitol dehydrogenase (SDH, EC 1.1.1.14) was immobilized in 40% yield in the presence of D-sorbitol (20 mM) and NAD (2 mM) with 10 mg of enzyme/g of PAN-800. Enzymatic analyses were carried out according to standard methods[14] with use of horse liver alcohol dehydrogenase for α-hydroxyaldehyde, glycerophosphate dehydrogenase (GPDH) for DHAP, and aldolase and GPDH for FDP.

Dihydroxyacetone phosphate (DHAP) was prepared enzymatically as described previously.[3]

L- and D-lactaldehydes were prepared, respectively, from D- and L-threonine by reaction with ninhydrin.[15] D,L-Lactaldehyde was prepared from pyruvaldehyde dimethyl acetal via reduction with $LiAlH_4$, followed by acid hydrolysis.[8]

D,L-α-Hydroxybutyraldehyde dimethyl acetal was prepared from butyraldehyde.[16] In a flask equipped with a stirrer and a reflux condenser was placed butyraldehyde (67 mL, 0.75 mol) and CH_2Cl_2 (30 mL). The solution was cooled to 10 °C in an ice bath, and a mixture of sulfuryl chloride (SO_2Cl_2, 72 mL, 0.75 mol) and 10 mL of CH_2Cl_2 was added over a period of 20 min; the reaction temperature was maintained between 15 and 40 °C. After completion of the addition, the reaction mixture was stirred at 25 °C for 1 h and refluxed for another hour. The mixture was then distilled through a 20-cm Vigreux column, and the fractions with bp 104–110 °C containing α-chlorobutyraldehyde were collected (44 g, 56% yield).

To 90 mL of dry methanol was added slowly 7.4 g (0.32 mol) of sodium metal. After reaction of the sodium had been completed, the temperature was maintained below 20 °C and 35 g (0.33 mol) of freshly prepared α-chlorobutyraldehyde was added. The mixture was stirred at room temperature for 1 h. The salts were separated by centrifugation and the supernatent was distilled to give 20 g (46% yield) of α-hydroxybutyraldehyde dimethyl acetal; bp 39–42 °C (1 mmHg) (lit.[16] bp 39–42 °C (1 mmHg)).

D,L-α-Hydroxybutyraldehyde. To a solution containing glacial acetic acid (15 mL), water (20 mL), and formic acid (0.5 mL) was added 8 g (60 mmol) of the α-hydroxybutyraldehyde dimethyl acetal prepared above. The mixture was incubated in a boiling water bath for 30 min and then concentrated under reduced pressure to an oily residue (8.1 g) that contained 59 mmol of α-hydroxybutyraldehyde (determined enzymatically with horse liver alcohol dehydrogenase and NADH). This material was used directly without further purification in aldol reactions.

Purification of Fructose 1,6-Diphosphate Dicalcium Salt ($FDPCa_2$). Technical grade $FDPCa_2$ (20 g, 63% purity, 30 mmol of FDP) in water (200 mL) was treated with Dowex 50 (400 g, H^+ form) to remove calcium ion. After filtration and washing with water (100 mL), the solution was passed through Dowex 1 (400 g, OH^- form) packed in a sintered glass filter, and the packed

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Table I. ^{13}C and ^{1}H Chemical Shifts of 6-Deoxy-D-fructose and 6-Deoxy-L-sorbose[a]

compd	^{13}C C_1	C_2	(C_3, C_4, C_5)[b]			C_6	^{1}H, CH_3
6-DeoxyF α[c]	68.91	106.45	85.13	78.27	83.24	21.52	1.32
β	65.62	103.65	78.92	77.92	82.04	21.61	1.30
6-DeoxyS α[c]	66.18	104.47	79.27	77.48	79.27	16.63	1.16
β	65.17	107.86	82.93	78.39	80.75	17.49	1.27

[a] Both are expressed in ppm downfield from DSS with D_2O as solvent at ambient temperature. [b] Not specifically assigned. [c] α and β forms are assigned on the basis of single-crystal X-ray data (Swaminathan, P.; Anderson, L.; Sundaralingam, M. *Carbohydr. Res.* **1979**, *75*, 1-10) and of those reported previously for 6-deoxyS in solution (Angyal, S. J.; Bethell, G. S. *Aust. J. Chem.* **1976**, *29*, 1249-65).

beads containing adsorbed FDP were washed with aqueous HCl (40 mM, 2.5 L). The FDP was eluted from the resin with 0.2 M HCl–0.1 M NaCl (1 L). Barium chloride (90 mmol) was added to the collected solution and the pH was adjusted to 8.0 by adding 5 N NaOH solution. Ethanol (500 mL) was added to the solution at 4 °C and the precipitated material was collected (28 g). Enzymatic analysis indicated the material contained 86% $FDPBa_2$ (24.6 mmol, 82% yield).

FDP from Fructose. To a 10-mL solution containing Tris (0.1 M, pH 7.6), $MgCl_2$ (10 mM), fructose (0.1 M), and ATP (0.2 M) was added HK (50 U) and PFK (50 U), and the mixture was kept at room temperature for 24 h. Enzymatic analysis indicated that FDP was produced (97 mM, 97% yield).

6-Deoxy-D-fructose 1-Phosphate (6-DeoxyF-1-P). A 100-mL solution containing FDP (20 mmol, sodium salt) and D-lactaldehyde (25 mmol), pH 7.0, was deoxygenated with argon. Aldolase (500 U in 15 mL of PAN-800 gel) and TPI (200 U in 1 mL of gel) was added to the solution, and the mixture was stirred at room temperature under argon. Enzymatic analysis for FDP showed that the reaction was complete in 2.5 days. The solution, after removal of the gel, was mixed with $BaCl_2$ (4.5 mmol) and the pH adjusted to 7.8 at 4 °C. Acetone (1 L) was added to the solution at 5 °C to obtain a precipitate (14.8 g) that contained 6-deoxyF-1-P barium salt (34 mmol determined with use of aldolase and GPDH, 87% yield): ^{1}H NMR (250 MHz, D_2O) δ 1.33 and 1.35 (d each, 3 H), 3.6–4.2 (m, 5 H). The activities of aldolase and TPI recovered were 78% and 75%, respectively, of the original values.

6-Deoxy-L-sorbose 1-phosphate (6-deoxyS-1-P) was prepared as its barium salt in the same way as that described above except that L-lactaldehyde was used instead of D-lactaldehyde, and an 80% yield with 86% purity was obtained: ^{1}H NMR (250 MHz, D_2O) δ 1.18 and 1.29 (d each, 3 H), 2.6–4.5 (m, 5 H) downfield from DSS.

Mixture of 6-deoxyF-1-P and 6-deoxyS-1-P was prepared similarly from a 1-L solution containing FDP (0.2, mol prepared by treatment of the Ca salt with Dowex 50 (H^+)), D,L-lactaldehyde (0.3 mol), $MgCl_2$ (5 mmol), and aldolase (100 U in 25 mL of gel) and TPI (500 U in 3 mL of gel) at pH 7.0. The reaction was complete in 2.5 days, and the product was isolated as its barium salt (152 g, 328 mmol) in 82% yield (82% purity determined enzymatically). The activities of aldolase and TPI recovered were 75% and 75%, respectively.

A similar reaction was carried out with DHAP as reactant. To a solution (1 L, pH 7.0) containing DHAP monopotassium salt[3] (0.2 mol) and D,L-lactaldehyde (0.21 mol) was added the aldolase (750 U) recovered from the above reaction. The mixture was kept under argon with stirring for 20 h to complete the reaction. The solution containing 184 mmol of 6-deoxyF-1-P and 6-deoxyS-1-P was used directly for the preparation of 6-deoxyF and 6-deoxyS (see below).

6-Deoxy-D-glucitol was prepared from 6-deoxyglucose and $NaBH_4$ in a procedure similar to that for the preparation of alditol.[17] A solution of 6-deoxyglucose[18] (0.25 g, Sigma) in H_2O (1 mL) was added to $NaBH_4$ (50 mg) suspended in water (1 mL) at 0 °C. The mixture was stirred at room temperature for 30 min. Dowex 50 (H^+ form) was added to the mixture until the generation of H_2 ceased. The mixture (pH ~4.0) was filtered and evaporated. Methanol (20 mL) was added to the residue and the mixture concentrated in vacuo to remove methyl borate. The residue (0.23 g, 90% yield) showed a single peak on HPLC with 85% CH_3CN as solvent (retention time: 5 min). ^{1}H NMR showed the characteristic resonance (1.24 ppm) of the methyl group; no aldehyde proton was observed.

Sorbitol Dehydrogenase (SDH) Catalyzed Oxidation of 6-Deoxy-D-glucitol. To a solution (200 mL) containing 6-deoxyglucitol (3.3 g, 20 mmol, 0.1 M), NAD (1 mM), α-ketoglutarate monosodium salt (4.2 g, 25 mmol), and $(NH_4)_2SO_4$ (3.3 g, 25 mmol) were added PAN-immobilized SDH (10 U in 5 mL of gel) and GluDH (40 U in 2 mL of gel). The pH was controlled automatically at 8.2 by addition of aqueous NaOH (1 N) through a peristaltic pump. The reaction was monitored by HPLC analysis (retention time: 6-deoxyF, 3.8 min; 6-deoxysorbitol, 5 min) and was complete in 10 h. The solution, after removal of the enzyme-containing gel, was concentrated to an oily residue, mixed with 20 mL of ethanol, and filtered to remove the precipitated material. The ethanol solution was concentrated again to an oily residue (3.1 g, 95% yield) that was homogeneous by HPLC and that had the same retention time as authentic 6-deoxyF: ^{1}H NMR (270 MHz, D_2O) δ 1.29 and 1.32 (d each, 3 H), 3.5–4.1 (m, 5 H). The TN and recovered activities for the active enzymes and cofactor were as follows: SDH, 2×10^6 (88%); GluDH, 7×10^6 (92%); NAD, 1000 (92%).

Amberlite IRA-400 Catalyzed Aldol Condensation. To a solution (50 mL) containing dihydroxyacetone (4.5 g, 50 mmol) and D,L-lactaldehyde (4.5 g, 50 mmol) was added Amberlite IRA-400 (OH^- form, 5 g). The mixture (pH 8.5) was stirred at room temperature for 10 min and filtered to remove the resin, and the filtrate was concentrated under reduced pressure to an oily residue (8.1 g, 90% yield). HPLC analysis showed two major components with the same retention times as 6-deoxyF (3.8 min) and 6-deoxyS (3.3 min) prepared in aldolase-catalyzed reactions. The ratio of 6-deoxyF to 6-deoxyS was 2 (determined on the basis of the peak height of the HPLC trace).

***O*-Methylglucoside 6-Phosphate.** The barium salt of glucose 6-phosphate (10 mmol) suspended in methanol (40 mL) was mixed with dried Dowex 50 (H^+ form, 5 g; the moisture had been removed by washing repeatedly with anhydrous methanol) and the mixture refluxed for 20 h. The mixture was filtered and the filtrate was concentrated to remove methanol. The product was not a substrate for G-6-PDH. The ^{1}H NMR spectrum showed no aldehyde proton. An aliquot of this material was dissolved in 1 N HCl and heated on a steam bath for 30 min. The hydrolysate was again a substrate for G-6-PDH. The yield, calculated from the change of absorbance at 340 nm, was 87% on the basis of G-6-P barium salt as starting material.

Removal of the Phosphate Group. The barium salt of 6-deoxyF-1-P or 6-deoxyS-1-P (4.4 g, 10 mmol) prepared above was suspended in H_2O (50 mL) and the mixture treated with Dowex 50 (H^+ form, 10 g) to remove barium ion. The solution (pH 1.0) obtained after filtration was heated at 90 °C for 8 h. Dowex 1 (HCO_3^- form) was then added to the solution at 25 °C with stirring to raise the pH to 5–6. The mixture was filtered and the filtrate concentrated to remove water. A mixture of methanol (10 mL) and acetone (10 mL) was added to the residue and the resulting precipitate discarded. The solution was concentrated to an oily residue (1.6 g, 85% yield) that showed single peak by HPLC. The

(17) Malfrom, M. L.; Thompson, A. *Methods Carbohydr. Chem.* **1963**, *2*, 65–8.

(18) Available from Sigma. It can be prepared from glucose by following the procedures outlined in Scheme II (Bollenback, G. N. *Methods Carbohydr. Chem.* **1963**, *2*, 326–8; Evans, M. E.; Parrish, F. W. *Ibid.* **1972**, *6*, 177–9; and ref 11).

deoxy sugar obtained was not a substrate for aldolase. Both ^{13}C and ^{1}H chemical shifts of compounds 6-deoxyF and 6-deoxyS are shown in Table I.

6,7-Dideoxyheptoses. DHAP (50 mmol) and D,L-α-hydroxybutyraldehyde (50 mmol) were subjected to aldolase-catalyzed condensation (200 U in 20 mL of gel) in a 1-L solution, pH 6.5, over 10 h, and a solution containing compounds **2** and **3** (45 mmol, determined by aldolase) was obtained. Unexceptional acid hydrolysis removed the phosphate moiety, and a mixture of 6,7-dideoxyheptoses was obtained (16 g, 82% overall yield).

Following the procedure described above for the Amberlite-catalyzed aldol condensation, dihydroxyacetone (4.5 g, 50 mmol) and D,L-α-hydroxybutyraldehyde (4.4 g, 50 mmol) were condensed to give a mixture of products (6.9 g) containing 6,7-dideoxyheptoses on the basis of HPLC analysis. The overall yield for the conversion was 78%.

Furaneol. To a solution containing 6-deoxyF or 6-deoxyS (2 g, 12.2 mmol) and ethanol (5 mL) in a glass tube were added piperidine (0.6 g) and glacial acetic acid (1 g). The tube was capped with a no-air stopper and the mixture was heated at 80 °C for 20 h. After removal of ethanol and acetic acid by concentration under reduced pressure, the residue was extracted into chloroform (160 mL) and washed three times with saturated NaCl solution (20 mL each time). The chloroform solution was concentrated under reduced pressure to an oily residue, which was dried in vacuo over concentrated H_2SO_4 and KOH pellets over night. The light brown material (1.2 g, 78% crude yield) obtained was recrystallized from petroleum ether, and pure Furaneol (1 g) was obtained: mp 79–80 °C dec (lit.[6] mp 78–80 °C dec); ^{1}H NMR (250 MHz) $CDCl_3$ containing Me_4Si internal standard) δ 1.40 (d, 3 H), 2.20 (s, 3 H), 4.50 (q, 1 H).

The following buffer components were tested (at 90 °C, 20 h) and no Furaneol was observed: acetic acid/ethanol, phosphate in H_2O (pH 3–4), phosphate in H_2O (pH 8.0), sodium bicarbonate in H_2O (pH 8.0), Dowex 1 acetate in H_2O, and ammonium acetate in ethanol.

Furaneol Analogues. A mixture of compounds **6** and **7** was prepared from 6,7-dideoxyheptoses with use of conditions similar to those for the preparation of Furaneol. Since **6** and **7** are not crystalline, isolation was accomplished by chromatography on silica gel column (2 × 50 cm) and eluted with organic solvent ($CHCl_3$/ethyl acetate = 10:1 v/v). Starting from a mixture of compounds **4** and **5** prepared via aldolase-catalyzed condensation, the yield of **6** and **7** was 50%. A 30% yield was obtained when starting from the compounds prepared via Amberlite-catalyzed condensation: ^{1}H NMR for **6** (270 MHz, $CDCl_3$) δ 0.99 (t, 3 H), 1.75 (m, 2 H), 4.37 (t, 1 H), 2.26 (s, 3 H); ^{1}H NMR for **7** (270 MHz, $CDCl_3$) δ 0.88 (t, 3 H), 2.63 (q, 2 H), 4.46 (q, 1 H), 1.45 (d, 3 H). The ratio of **6** to **7** is 7:2.

Hydrogenolysis of FDP. A 200-mL pressure bottle was charged with $FDPCa_2$ (75% pure, 18 mmol, 10 g), NaI (8.9 mmol, 1.34 g), MeOH (50 mL), and distilled water (50 mL). KOH (31.3 mmol, 1.76 g) was also introduced to the rapidly stirred mixture. With 1.0 g of KOH, the pH was 12. With 1.76 g, the pH was 14. Pd/C (10%, 200 mg) was added to the bottle, which was then closed, flushed, and pressurized at 60 psi with hydrogen. After 24 h at 90 °C, the pH was 7. The catalyst and insoluble inorganic phosphate were filtered, and the yellow solution was extracted. After workup, a yellow oil was obtained (660 mg, 28.7%). Most of the oil crystallized overnight on standing at 4 °C under argon. The crystalline solid was Furaneol (mp 77–79 °C); spectral characteristics were indistinguishable from those of an authentic sample.

When the reaction was done with a buffer and a lower pressure of hydrogen, a 20-mL pressure bottle was charged with piperidine (1.2 mmol, 102 mg), absolute MeOH (3 mL), glacial acetic acid (2.7 mmol, 162 mg), distilled water (3 mL), $FDP{\cdot}Na_3$ (98% pure, 2.09 mmol, 850 mg), and 10% Pd/C (10 mg). A magnetic stirring bar was placed in the container, which was closed, flushed with argon and hydrogen, and finally pressurized at 15 psi of hydrogen.

Furaneol from 6-Deoxy-D-glucose. A 5-mL round-bottomed flask was charged with 6-deoxy-D-glucose (0.913 mmol, 150 mg) and a 2-mL ethanolic solution of glacial acetic acid (1.25 mmol, 75 mg) and piperidine (0.53 mmol, 45 mg). A magnetic stirring bar was placed in the flask, and the flask was closed with a septum and flushed with argon. The reaction was carried out at 80 °C for 24 h. After the transformation was complete, the yellow solution was extracted with chloroform (four times) and NaCl-saturated water. The organic phase was dried over $MgSO_4$, the solution filtered, and the solvent removed at reduced pressure to give 110 mg of Furaneol (90% pure, 85% yield).

Registry No. **1**, 71075-63-3; **2**, 86943-33-1; **3**, 86943-34-2; **4**, 86953-24-4; **5**, 86953-25-5; **6**, 27538-10-9; **7**, 27538-09-6; I, 3658-77-3; aldolase, 9024-52-6; TPI, 9023-78-3; SDH, 9028-21-1; G-6-P, 56-73-5; G-1-P, 59-56-3; Gal-6-P, 6665-00-5; F-1-P, 15978-08-2; F-6-P, 643-13-0; 6-deoxy-F-1-P, 86992-64-5; 6-deoxy-S-1-P, 86992-65-6; 6-deoxy-F, 4429-06-5; 6-deoxy-S, 18545-94-3; FDP, 488-69-7; DHAP, 57-04-5; DL-α-hydroxybutyraldehyde, 86943-35-3; L-lactaldehyde, 3913-64-2; D-lactaldehyde, 3946-09-6; 6-deoxy-D-glucose, 7658-08-4; butyraldehyde, 123-72-8; α-chlorobutyraldehyde, 28832-55-5; DL-α-hydroxybutyraldehyde dimethyl acetal, 86943-36-4; DHA, 96-26-4; DL-lactaldehyde, 3913-65-3; Amberlite IRA-400, 9002-24-8; D-fucose, 3615-37-0; L-fucose, 2438-80-4.

Supplementary Material Available: Detailed experiments regarding the preparation of Furaneol from fucose and different hexose phosphates by hydrogenolysis (5 pages). Ordering information is given on any current masthead page.

C(12)-Substituted Prostaglandins. An Enantiospecific Total Synthesis of (+)-12-(Fluoromethyl)prostaglandin $F_{2\alpha}$ Methyl Ester

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Received April 8, 1983

(+)-12-(Fluoromethyl)prostaglandin $F_{2\alpha}$ methyl ester (**1**) was prepared from the readily available methyl (−)-(1α,4α,5α,7*S**)-5-bromospiro[bicyclo[2.2.1]heptane-2,2′-[1,3]dioxolane]-7-carboxylate (**2**). The synthesis proceeds via the intermediacy of aldehyde **10** (R = CH_2F), which upon reaction with 1-lithio-1-*cis*-heptene provides in an enantiospecific process access to adduct **11** (R = CH_2F). Exposure of the desired cis-allylic acetate **12** to $PdCl_2(CH_3CN)_2$ in tetrahydrofuran gives rise solely to enantiomerically pure trans-allylic acetate **14**, which is transformed into **1** via standard prostaglandin methodology. (+)-12-(Fluoromethyl)prostaglandin $F_{2\alpha}$ methyl ester was evaluated for pregnancy interruption in the hamster and smooth muscle stimulating properties on gerbil colon and hamster uterine strips.

Several years ago we embarked on a program to prepare a variety of C(12)-substituted prostaglandin derivatives for evaluation as potential luteolytic agents. At the time we initiated our program, synthetic routes to C(12)-substituted prostaglandins were lacking. Despite the development during the past 10 years of methodology for gaining access to the C(12) position in the prostaglandin nucleus,[1] C(12)-derivatized prostaglandins remain rela-

0022-3263/83/1948-3497$01.50/0