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Microbial Synthesis of the Energetic Material Precursor 1,2,4-Butanetriol

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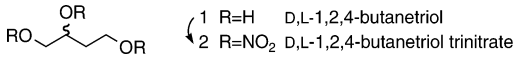
Nitration of racemic D,L-1,2,4-butanetriol **1** affords the energetic material D,L-1,2,4-butanetriol trinitrate **2**, which is less shock sensitive, more thermally stable, and less volatile than nitroglycerin.[1] However, the limited availability of 1,2,4-butanetriol has impeded substitution of 1,2,4-butanetriol trinitrate for nitroglycerin. Catalytic hydrogenation of D,L-malic acid (Scheme 1) constitutes one synthetic route to D,L-1,2,4-butanetriol. This account establishes microbial synthesis as an alternative catalytic strategy. Enzymes from three different microbes are recruited to create biosynthetic pathways (Scheme 2) by which D-1,2,4-butanetriol **1a** and L-1,2,4-butanetriol **1b** are derived from D-xylose **4a** and L-arabinose **4b**, respectively.



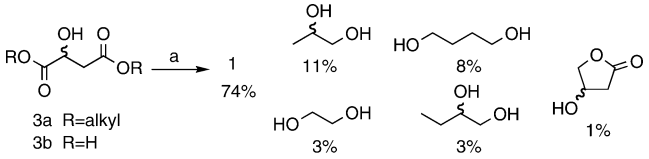
Commercial synthesis[2] of D,L-1,2,4-butanetriol employs $NaBH_4$ reduction of esterified D,L-malic acid **3**.[3] For every ton of 1,2,4-butanetriol synthesized, multiple tons of byproduct borates are generated. D,L-Malic acid can also be hydrogenated over various catalysts (Cu−Cr, Cu−Al, Ru−Re) at 2900−5000 psi of H_2 and 60−160 °C reaction temperatures.[4] Yields of 1,2,4-butanetriol range from 60% to 80%. A variety of byproducts are also formed during high-pressure hydrogenation.[4] These byproducts are not generated when esterified malic acid is reduced using $NaBH_4$.[3] D,L-Malic acid is synthesized from the *n*-butane component of liquefiable petroleum gas via intermediacy of maleic anhydride.[5]

To examine product and byproduct yields in detail, Ru on C was selected as the catalyst for reduction of malic acid due to its commercial availability and use in lactic acid hydrogenations.[6] Reduction of D,L-malic acid was optimized relative to H_2 pressure, temperature, concentration, and the ratio of catalyst to substrate. Hydrogenation at 5000 psi and 135 °C of a 1 M aqueous solution of malic acid using 1.3 mol % relative to substrate of 5 wt % Ru on C afforded 1,2,4-butanetriol in 74% yield. Byproducts (Scheme 1) accounted for a total of 25% of the starting malic acid and complicated purification of D,L-1,2,4-butanetriol using distillation.

Melting points, a key consideration in energetic material formulations, differ for a single enantiomer relative to a racemic mixture. Microbial syntheses were therefore needed for both 1,2,4-butanetriol enantiomers.[7] To address this challenge, the opposing C-4 stereogenic centers of D-xylose and L-arabinose were exploited (Scheme 2) as the basis for synthesis of D-1,2,4-butanetriol **1a** and L-1,2,4-butanetriol **1b**, respectively. Both D-xylose and L-arabinose are abundantly available in heteroxylans derived from corn fiber and sugar beet pulp.[8] Mixing of microbe-synthesized enantiomers **1a** and **1b** (Scheme 2) would provide the equivalent of the racemic D,L-1,2,4-butanetriol currently used to manufacture D,L-1,2,4-butanetriol trinitrate.

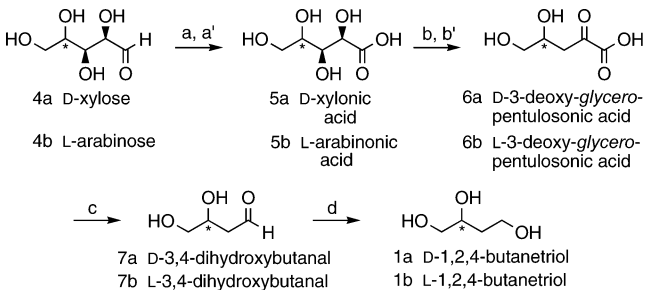
Microbial synthesis (Scheme 2) of 1,2,4-butanetriol enantiomers began with the reported oxidation of D-xylose using fermentor-controlled cultures (1 L scale) of *Pseudomonas fragi* ATCC4973.[9] D-Xylose (100 g/L) was oxidized at 30 °C to D-xylonic acid **5a** (77 g/L) in 70% yield. L-Arabinose was discovered to be similarly oxidized in 54% overall yield to a mixture of L-*arabino*-1,4-lactone (40 g/L) and L-arabinonic acid **5b** (15 g/L). The lactone was subsequently hydrolyzed to L-arabinonic acid. *Escherichia coli* constructs were then employed for the conversion of D-xylonic **5a** acid and L-arabinonic acid **5b** into the enantiomers of 1,2,4-butanetriol.

Scheme 1 [a]



[a] Reaction conditions: 5000 psi H_2, Ru on C, 135 °C, H_2O.

Scheme 2 [a]



[a] Enzymes (microbial source): (a) D-xylose dehydrogenase (*P. fragi*); (a′) L-arabinose dehydrogenase (*P. fragi*); (b) D-xylonate dehydratase (*E. coli*); (b′) L-arabinonate dehydratase (*P. fragi*); (c) benzoylformate decarboxylase (*P. putida*); (d) dehydrogenase (*E. coli*).

Previously undocumented catabolism of D-xylonic acid by *E. coli* K-12 was discovered to coincide with D-xylonate dehydratase[10] expression. Transport of D-xylonic acid and its conversion into 3-deoxy-D-*glycero*-pentulosonic acid **6a** (Scheme 2) thus employed enzymes native to *E. coli*. L-Arabinonic acid catabolism and L-arabinonate dehydratase[9b,11] activity needed for generation of 3-deoxy-L-*glycero*-pentulosonic acid **6b** (Scheme 2) were absent in *E. coli*. This necessitated the isolation and heterologous expression of the encoding genes from a *P. fragi* genomic DNA library. Three cosmids enabled *E. coli* K-12 to catabolize L-arabinonic acid. From a 5.0 kb region shared between these cosmids, *aadh*-encoded L-arabinonate dehydratase and an *aatp*-encoded L-arabinonate transport protein were identified.

Attention then turned to identification of a 2-ketoacid decarboxylase capable of catalyzing the conversions of pentulosonic acid **6a** to D-3,4-dihydroxybutanal **7a** and pentulosonic acid **6b** to L-3,4-dihydroxybutanal **7b** (Scheme 2). Only benzoylformate decarboxylase expressed by *Pseudomonas putida*[12a] catalyzed these reactions. Other 2-ketoacid decarboxylases screened but found to lack the requisite activity included indole 3-pyruvate decarboxylase expressed by *Erwinia herbicola*[12b] and a variety of different pyruvate

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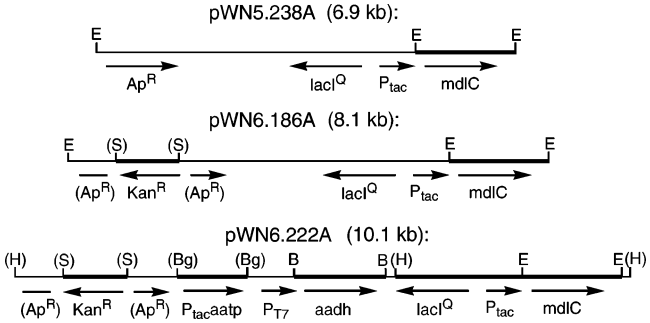
Scheme 3 [a]



[a] Plasmids (size): restriction enzyme maps. Sites are abbreviated as follows: B = *Bam*HI, Bg = *Bgl*II, E = *Eco*RI, H = *Hin*dIII, S = *Sca*I. Parentheses indicate that the designated enzyme site has been eliminated. Lightface lines indicate vector DNA; boldface lines indicate insert DNA.

decarboxylases expressed by *Zymomonas mobilis*,[12c] *Acetobacter pasteurianus*,[12d] *Zymobacter palmae*,[12e] and *Saccharomyces cerevisiae*.[12f]

Native dehydrogenase activity in *E. coli* was anticipated to be adequate for the reduction of butanal **7a** to D-1,2,4-butanetriol **1a** and butanal **7b** to L-1,2,4-butanetriol **1b** (Scheme 2). To test for the needed dehydrogenase activity, intact *E. coli* DH5α/pWN5.238A (Scheme 3) expressing benzoylformate decarboxylase was incubated in medium containing racemic D,L-3-deoxy-*glycero*-pentulosonic acid. Accumulation of D,L-1,2,4-butanetriol indicated that *E. coli* expressed the required dehydrogenase activity under aerobic culture conditions.

With the required enzyme activities identified, *E. coli* constructs were assembled for the conversions of D-xylonic acid **5a** and L-arabinonic acid **5b** synthesized by *P. fragi* from D-xylose **4a** and L-arabinose **4b** (Scheme 2). D-1,2,4-Butanetriol-synthesizing *E. coli* DH5α/pWN6.186A (Scheme 3) carried a *P. putida mdlC* plasmid insert encoding benzoylformate decarboxylase while relying on native D-xylonate transport along with native D-xylonate dehydratase and dehydrogenase activities. The required heterologous expression of only a single gene was a consequence of *E. coli* catabolism of D-xylonic acid. By contrast, L-1,2,4-butanetriol-synthesizing *E. coli* BL21(DE3)/pWN6.222A (Scheme 3) carried the *P. putida mdlC* plasmid insert encoding benzoylformate decaboxylase, a *P. fragi aadh* plasmid insert encoding L-arabinonate dehydratase, and a *P. fragi aatp* insert encoding an L-arabinonate transport protein. Alcohol dehydrogenase activity was the only native *E. coli* enzyme activity recruited for L-1,2,4-butanetriol synthesis.

Fermentor-controlled cultivation (1 L) of *E. coli* DH5α/pWN6.186A at ambient pressure and 33 °C resulted in the conversion of D-xylonic acid (10 g/L) into D-1,2,4-butanetriol (1.6 g/L) in 25% yield. Similar cultivation of *E. coli* BL21(DE3)/pWN6.222A led to the conversion of L-arabinonic acid (10 g/L) into L-1,2,4-butanetriol (2.4 g/L) in 35% yield. Stereochemical assignments for microbe-synthesized products were based on the conversion to Mosher esters and comparison with similarly derivatized D- and L-1,2,4-butanetriol obtained from commercial sources.[13] *E. coli* DH5α/pWN6.186A synthesized ethylene glycol (0.093 g/L) for a 3% yield of this byproduct, while *E. coli* BL21(DE3)/pWN6.222A synthesized ethylene glycol (0.087 g/L) in 2% yield.

A key feature of the microbial synthesis of 1,2,4-butanetriol is the substitution of a straightforward enzymatic reduction of an aldehyde for the problematic catalytic reduction of a carboxylic acid. The high H_2 pressures and elevated temperatures required for hydrogenation of malic acid are thus avoided. Byproduct formation resulting from cleavage of carbon−carbon bonds is also substantially reduced. Further metabolic engineering is clearly required to increase product yields and concentrations. Nonetheless, microbial catalysis is an intriguing alternative to catalytic hydrogenation for the large-scale synthesis of 1,2,4-butanetriol needed for replacement of nitroglycerin with 1,2,4-butanetriol trinitrate. The significance of such a substitution is considerable given that nitroglycerin has been used in industrial and military energetic materials since the original dynamite formulations developed by Nobel.[14]

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Supporting Information Available: Hydrogenation of malic acid; isolation of *aadh* and *aatp*; enzyme assays; microbial oxidation of D-xylose and L-arabinose; microbial synthesis of D- and L-1,2,4-butanetriol; enantiomer analysis (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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