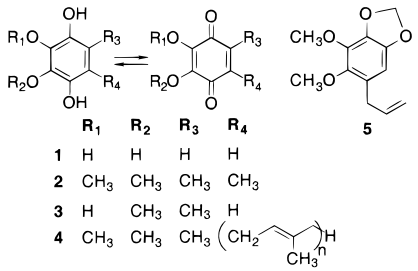
Synthesis of 1,2,3,4-Tetrahydroxybenzene from D-Glucose: Exploiting *myo*-Inositol as a Precursor to Aromatic Chemicals

Chad A. Hansen, Amy B. Dean, K. M. Draths, and J. W. Frost*

Department of Chemistry, Michigan State University East Lansing, Michigan 48824

Received November 23, 1998

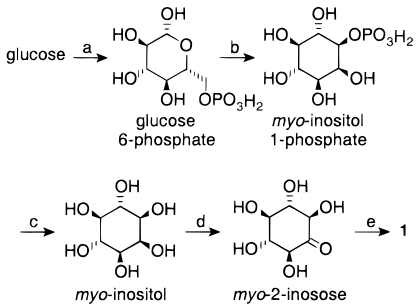
Polyhydroxy benzenes and quinones possessing the oxygenation pattern of 1,2,3,4-tetrahydroxybenzene **1** often display biological activity. Aurantiogliocladin **2** and fumigatin **3** are antibiotics.[1] Coenzyme $Q_{n=10}$ **4** is an essential antioxidant in humans protecting low-density lipoproteins from atherosclerosis-related oxidative modification.[2] Dillapiole **5** is a pyrethrin synergist and responsible for the sedative effect of *Perilla frutescens* leaves.[3] A synthetic route (Scheme 1) has now been elaborated which provides convenient access to 1,2,3,4-tetrahydroxybenzene via *myo*-inositol intermediacy. The general utility of this route is demonstrated by a concise synthesis of coenzyme $Q_{n=3}$ **4**. While the shikimate pathway and polyketide biosynthesis have traditionally provided biocatalytic access to aromatic chemicals, syntheses of 1,2,3,4-tetrahydroxybenzene **1** and coenzyme $Q_{n=3}$ **4** are distinguished by the recruitment of *myo*-inositol biosynthesis.



Synthesis of *myo*-inositol by *Escherichia coli* JWF1/pAD1.88A begins with D-glucose uptake and conversion to D-glucose 6-phosphate catalyzed by the *E. coli* phosphotransferase system[4] where phosphoenolpyruvate is the source of the transferred phosphoryl group (Scheme 1). D-Glucose 6-phosphate then undergoes cyclization to *myo*-inositol 1-phosphate catalyzed by *myo*-inositol 1-phosphate synthase. This enzyme activity, which results from expression of the *Saccharomyces cerevisiae INO1* gene[5] on plasmid pAD1.88A, varied significantly (0.022, 0.043, 0.018, and 0.009 μmol/min/mg at 18, 30, 42, and 54 h, respectively) over the course of the fermentation.

E. coli JWF1/pAD1.88A synthesized 21 g/L *myo*-inositol and 4 g/L *myo*-inositol 1-phosphate in 11% combined yield (mol/mol) from D-glucose under fed-batch fermentor conditions (Figure 1). Both *myo*-inositol and *myo*-inositol 1-phosphate accumulated in the culture supernatant. In eucaryotes, hydrolysis of *myo*-inositol 1-phosphate to *myo*-inositol is catalyzed by the enzyme inositol monophosphatase.[6] Phosphoester hydrolysis was fortuitously catalyzed in *E. coli* JWF1/pAD1.88A by unidentified cytosolic or periplasmic phosphatase activity.

Scheme 1[a]



[a] Key: (a) phosphoenolpyruvate:carbohydrate phosphotransferase; (b) *myo*-inositol 1-phosphate synthase; (c) phosphatase activity; (d) dehydrogenase activity; (e) 0.5 M H_2SO_4, H_2O, reflux.

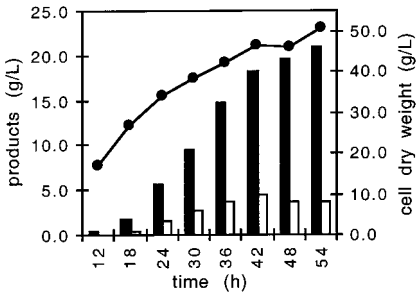


Figure 1. Cultivation of *E. coli* JWF1/pAD1.88A under fed-batch fermentor conditions: solid bar, inositol; open bar, *myo*-inositol 1-phosphate; (●) cell dry weight.

Oxidation of *myo*-inositol to *myo*-2-inosose, the next step in the conversion of D-glucose into 1,2,3,4-tetrahydroxybenzene **1**, is the first catabolic step when *myo*-inositol is used as a sole source of carbon for growth and metabolism by microbes such as *Bacillus subtilis*.[7] *myo*-Inositol can also be oxidized by *Gluconobacter oxidans* without loss of product *myo*-2-inosose to catabolism.[8] Accordingly, incubation of *G. oxidans* ATCC 621 in medium containing microbe-synthesized *myo*-inositol led to the formation of *myo*-2-inosose (Scheme 1) in 95% isolated yield.

Inososes have been thought to be stable under acidic conditions and reactive under basic conditions with reported aromatizations resulting from successive β-eliminations being dominated by formation of 1,2,3,5-tetrahydroxybenzene.[9] We, however, observed *myo*-2-inosose to be reactive under acidic conditions with no apparent formation of 1,2,3,5-tetrahydroxybenzene. Refluxing *G. oxidans*-produced *myo*-2-inosose for 9 h in degassed, aqueous 0.5 M H_2SO_4 under argon cleanly afforded 1,2,3,4-tetrahydroxybenzene in 66% isolated yield.

Conversion of D-glucose into 1,2,3,4-tetrahydroxybenzene **1** is a three-step synthesis. 1,2,3,4-Tetrahydroxybenzene **1** has historically been obtained from pyrogallol **6** by a longer route (Scheme 2) involving synthesis and subsequent hydrolysis of aminopyrogallol **7**.[10] Due to the tedious nature of this synthesis,[10b] two alternate routes (Scheme 2) were used to obtain authentic

(1) (a) Vischer, E. B. *J. Chem. Soc.* **1953**, 815. (b) Baker, W.; McOmie, J. F. W.; Miles, D. *J. Chem. Soc.* **1953**, 820. (c) Baker, W.; Raistrick, H. *J. Chem. Soc.* **1941**, 670.

(2) (a) Ingold, K. U.; Bowry, V. W.; Stocker, R.; Walling, C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 45. (b) Stocker, R.; Bowry, V. W.; Frei, B. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1646. (c) Steinberg, D. *Circulation* **1991**, *84*, 1420.

(3) (a) Honda, G.; Koezuka, Y.; Tabata, M. *Chem. Pharm. Bull.* **1988**, *36*, 3153. (b) Tomar, S. S.; Saxena, V. S. *Agric. Biol. Chem.* **1986**, *50*, 2115.

(4) Postma, P. W.; Lengeler, J. W.; Jacobson, G. R. In *Escherichia coli and Salmonella*, 2nd ed.; Neidhardt, F. C., Curtiss, R., III, Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M., Umbarger, H. E., Eds.; ASM: Washington, 1996; Vol. 1, p 1149.

(5) Dean-Johnson, M.; Henry, S. A. *J. Biol. Chem.* **1989**, *264*, 1274.

(6) McAllister, G.; Whiting, P.; Hammond, E. A.; Knowles, M. R.; Atack, J. R.; Bailey, F. J.; Maigetter, R.; Ragan, C. I. *Biochem. J.* **1992**, *284*, 749.

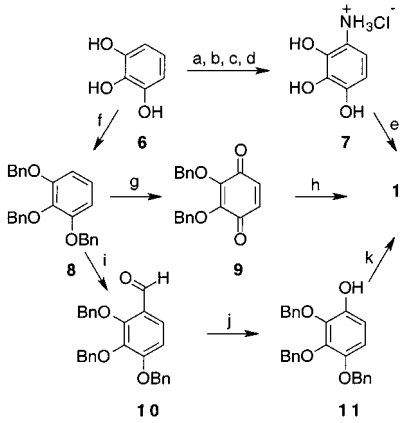
(7) Yoshida, K.-I.; Aoyama, D.; Ishio, I.; Shibayama, T.; Fujita, Y. *J. Bacteriol.* **1997**, *179*, 4591.

(8) Posternak, T. *Biochem. Prep.* **1952**, *2*, 57.

(9) (a) Posternak, T. *The Cyclitols*; Holden-Day: San Fransisco, 1965; Chapter 8. (b) Angyal, S. J.; Range, D.; Defaye, J.; Gadelle, A. *Carbohydr. Res.* **1979**, *76*, 121.

10.1021/ja9840293 CCC: $18.00
Published on Web 04/07/1999

Scheme 2[a]

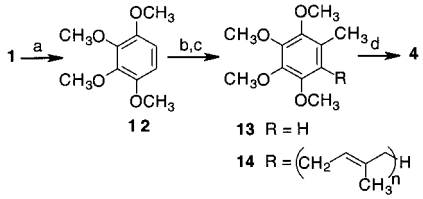


[a] Key: (a) $Cl_2C(O)$, pyridine, xylene, reflux; (b) H_2SO_4, HNO_3; (c) KOH (aq); (d) Zn, HCl; (e) H_2O, reflux; (f) BnBr, K_2CO_3, acetone, reflux, 83%; (g) $K_3Fe(CN)_6$, H_2O_2, AcOH, 11%; (h) H_2, 10% Pd/C, EtOH, 100%; (i) *N*-methylformanilide, $POCl_3$, 60 °C, 93%; (j) HCO_2H, H_2O_2, CH_2Cl_2, 0 °C to room temperature, 95%; (k) H_2, 10% Pd/C, EtOH, 80%.

samples of 1,2,3,4-tetrahydroxybenzene **1**. Low-yielding, direct hydroxylation[11] of protected pyrogallol **8** or higher-yielding, indirect oxidation via formyl **10** intermediacy[12] yielded respectively quinone **9** and phenol **11**.[12] Hydrogenation of **9** and **11** afforded products which were identical to 1,2,3,4-tetrahydroxybenzene **1** synthesized (Scheme 1) from D-glucose.

Variations in strategies employed for hydroxyl protection combined with the ease of metalation and alkylation of the aromatic nucleus makes **1** a versatile intermediate for the synthesis of a wide spectrum of naturally occurring 1,2,3,4-tetrahydroxybenzene derivatives. For example, permethylation (Scheme 3) of **1** leads to tetramethyl **12** which undergoes facile lithiation and methylation affording **13** in high yield. Formation of an organocuprate from **13**, farnesylation, and subsequent reaction with $(NH_4)_2Ce(NO_3)_6$ affords coenzyme $Q_{n=3}$ **4**. This four-step synthesis of coenzyme Q_n from tetrahydroxybenzene **1** is equal in length to the shortest reported[13a] synthesis of coenzyme Q_n which

Scheme 3[a]



[a] Key: (a) $(CH_3)_2SO_4$, NaOH, 69%; (b) (i) *n*-BuLi, TMEDA, hexanes, THF, 0 °C; (ii) CH_3I, 0 °C, 83%; (c) (i) *n*-BuLi, TMEDA, hexanes, 0 °C; (ii) CuCN, THF, Et_2O, 0 °C; (iii) farnesyl bromide, −78 °C, 57%; (d) CAN, pyridine-2,6-dicarboxylate, CH_3CN/H_2O, 0 °C, 46%.

uses *p*-cresol as a starting material and substantially shorter than syntheses of coenzyme Q_n from pyrogallol, gallic acid, or vanillin.[13b−d]

Only one oxygen atom in coenzyme Q_n, a shikimate pathway product, is directly derived from D-glucose. The remaining oxygen atoms are derived from O_2 via enzyme-catalyzed hydroxylations. Trihydroxybenzenes, pyrogallol and phloroglucinol possess the maximum number of oxygen atoms attached to a benzene nucleus by the shikimate pathway or polyketide biosynthesis in lieu of enzyme-catalyzed hydroxylation. At least a dozen enzymes are required to disassemble and reassemble the carbon atoms of D-glucose into the benzene nucleus of coenzyme Q_n, pyrogallols, and phloroglucinols. By comparison, synthesis of 1,2,3,4-tetrahydroxybenzene **1** via *myo*-inositol intermediacy requires only four enzymes and an acid-catalyzed dehydration for all six carbon and all four oxygen atoms to be directly derived from the carbon and oxygen atoms of D-glucose. Synthesis (Scheme 1) of 1,2,3,4-tetrahydroxybenzene **1** is thus a useful example of enzyme and atom[14] economy in organic synthesis in addition to being a significant strategic departure from previous biocatalytic syntheses of aromatic chemicals from D-glucose.

Acknowledgment. Research was supported by the National Science Foundation. Professor Susan A. Henry provided the *INO1* locus.

Supporting Information Available: Synthesis of *myo*-inositol, *myo*-2-inosose, **1**, **8**, **9**, **10**, **11**, **12**, **13**, **14**, and **4** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA9840293

(10) (a) Leston, G. In *Kirk-Othmer Encyclopedia of Chemical Technology*, 4th ed.; Kroschwitz, J. I., Howe-Grant, M., Eds.; Wiley: New York, 1996; Vol. 19, p 778. (b) Einhorn, A.; Cobliner, J.; Pfeiffer, H. *Ber. Dtsch. Chem. Ges.* **1904**, *37*, 110.

(11) Matsumoto, M.; Kobayashi, H.; Hotta, Y. *J. Org. Chem.* **1985**, *50*, 1766.

(12) Kolonits, P.; Major, Á.; Nógrádi, M. *Acta Chim. Hung.* **1983**, *113*, 367.

(13) (a) Keinan, E.; Eren, D. *J. Org. Chem.* **1987**, *52*, 3872. (b) Syper, L.; Kloc, K.; Mlochowski, J. *Tetrahedron* **1980**, *36*, 123. (c) Sugihara, H.; Watanabe, M.; Kawamatsu, Y.; Morimoto, H. *Liebigs Ann. Chem.* **1972**, *763*, 109. (d) For an overview of earlier syntheses, see: Mayer, H.; Isler, O. *Methods Enzymol.* **1971**, *18*, 182.

(14) Trost, B. M. In *Green Chemistry*; Anastas, P. T., Williamson, T. C., Eds.; Oxford: New York, 1998; Chapter 6.