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An expeditious chemo-enzymatic route from glucose to catechol by the use of 2-deoxy-*scyllo*-inososе synthase

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Abstract

A potential two-step process to catechol from D-glucose comprising one-pot incubation of D-glucose with recombinant 2-deoxy-*scyllo*-inososе synthase (BtrC) and hexokinase, together with chemical reductive dehydration of the resulting 2-deoxy-*scyllo*-inososе with HI, was developed.

Keywords: chemo-enzymatic route; catechol; carbocycle forming enzyme; glucose.

Facing the inevitable shortage of fossil resources in the coming centuries, it is highly desirable to facilitate new technologies for the production of versatile chemicals from sustainable and/or recyclable resources, preferably using environmentally acceptable methods.[1] Frost et al. demonstrated pioneering works for the production of catechol and other benzenoid chemicals by the use of the enzymes involved in the shikimate pathway as well as by *myo*-inositol synthase.[2]

During our work on the molecular analysis of the biosynthesis of clinically important 2-deoxystreptamine-containing aminocyclitol antibiotics,[3] we were successful in purifying the key enzyme, 2-deoxy-*scyllo*-inososе synthase (DOIS) from butirosin-producing *Bacillus circulans* SANK72073.[4] Subsequently, its structural gene (*btrC*) has been identified and heterologously over-expressed recently in *Escherichia coli*.[5] DOIS catalyzes the multi-step direct cyclization of D-glucose-6-phosphate (G-6-P) into the six-membered carbocycle 2-deoxy-*scyllo*-inososе (DOI) as illustrated in Scheme 1.

With over-expressed DOIS in hand, we envisioned that the DOI product may well be conveniently converted into catechol or other benzenoids by rather simple chemical reactions, because its unique unsymmetrical structure differs from *myo*-inositol.[2d] Catechol is an important industrial chemical used as an antioxidant, starting material for flavors and fragrances, medical and agricultural agents, and for other things. This communication concerns preliminary results of a novel chemo-enzymatic two-step

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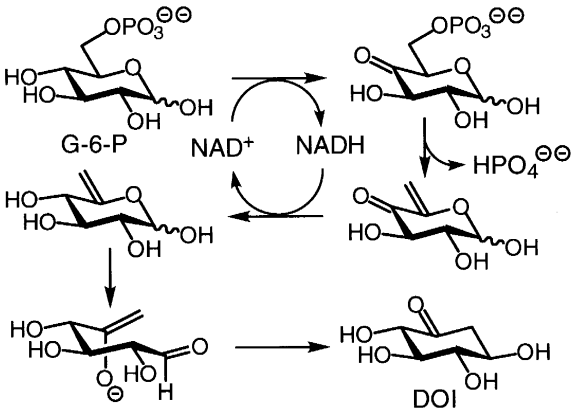
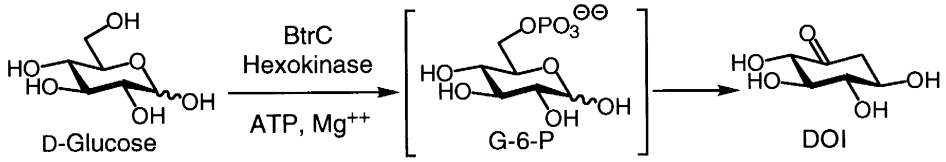


Scheme 1.

approach to catechol from D-glucose. In addition to this potential, DOI itself is an interesting chiral 2,3,4,5-tetrahydroxycyclohexanone resource for asymmetric synthesis.

In order to transform D-glucose to catechol or other benzenoids by way of DOI, two issues had to be addressed; one was phosphorylation of D-glucose to G-6-P as the substrate of DOIS (BtrC), and the other was dehydration of DOI to catechol.

As to the first requisite, it seemed logical and appropriate to use hexokinase in the presence of adenosine triphosphate (ATP) for the convenient preparation of G-6-P, since the enzyme is easily available and several procedures for the regeneration of the ATP cofactor have been well documented.[6] After several attempts, one-pot conversion of D-glucose to DOI turned out to be successful, as shown in Scheme 2. Thus, simultaneous incubation of D-glucose with commercially available hexokinase and the recombinant BtrC, the latter being over-expressed in *E. coli* and purified to homogeneity,[5] in the presence of ATP, nicotinamide adenine dinucleotide (NAD^+), and Co^{++}, yielded DOI in up to 38% yield. It should be pointed out that the yield may well be improved, for example, by the use of a larger amount of enzymes, since an independent incubation of G-6-P with recombinant BtrC was able to afford DOI almost quantitatively.[7]



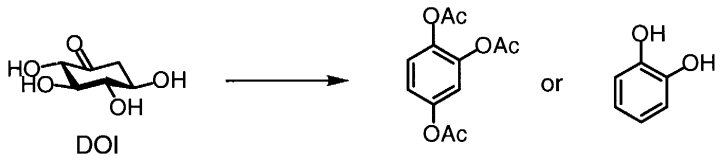
Scheme 2.

The most crucial was the second issue, since any such reactions, particularly a rather simple reaction, had not been known. Our efforts were devoted to this problem, and the results are summarized in Table 1.

1,2,4-Trihydroxybenzene was produced as its corresponding acetate under acidic acetolysis or acetylation conditions (runs 1, 2, and 3). Importantly, reductive dehydration (runs 4 and 5) clearly yielded catechol as a major product. Particularly, treatment of DOI with HI[8] allowed direct formation of catechol in good yield (59%). Thus, catechol can now be produced from DOI in a single chemical step, although the reaction conditions need to be further optimized.

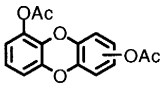
Typical procedures: One-pot synthesis of DOI from D-glucose by BtrC and hexokinase: A mixture (total volume 100 μl) of 10 mM D-glucose, purified BtrC (27 μM) and hexokinase (0.2 U/ml, Boehringer Mannheim) was incubated in the presence of 5 mM NAD^+ (Oriental Yeast Co., Tokyo), 10 mM ATP

Table 1



entry	condition	isolated yield (%) 1,2,4-$(OAc)_3$-benzene	catechol
1	Ac_2O, AcOH, conc. H_2SO_4, reflux	20[a]	—
2	Ac_2O, AcOH, reflux	29[b]	—
3	Ac_2O, Py, rt	26	—
4	Zn, AcOH, reflux	—[c]	18
5	conc. HI, AcOH, 70°C	—	59

a, dibenzo-*p*-dioxin type compounds (5%) were also isolated.
b, 1,2,3,4-$(OAc)_4$- (4%) and 1,2,3,5-$(OAc)_4$-benzene (4%) were also isolated.
c, formation of 1,2,4-$(OH)_3$-benzene was detected.



(Tokyo Kasei Kogyo TCI, Tokyo), 5 mM $MgCl_2$, and 0.1 mM $CoCl_2$ at 37°C for 11 h, and additional incubation was further continued at 46°C for 30 min. The yield was quantitated to be 38% by HPLC after the reaction product was derivatized to *O*-(4-nitrobenzyl)oxime.[3e]

Catechol from DOI: A mixture of 43 mg of 2-deoxy-*scyllo*-inosose, 4 ml of glacial acetic acid and 2.5 ml of conc. hydroiodic acid was heated under Ar at 70°C for 2 h. To the resulting reaction mixture was added an aqueous saturated $NaHSO_3$ solution and the whole was extracted four times with 70 ml each of ethyl acetate. The combined organic extract was dried over anhydrous $MgSO_4$. After being filtered, the extract was evaporated to dryness to give a residue, which was chromatographed on silica gel (Merck Kieselgel 60, Art. 7734) using hexane:ethyl acetate (8:1) as solvent to give 17 mg (59% yield) of catechol: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.87 (m, aromatic), 6.81 (m, aromatic), 5.08 (2×OH, br); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.5, 121.3, 115.5.

Catechol from G-6-P: A mixture (total volume 20 ml) of 5 mM G-6-P, purified BtrC (0.23 μM) was incubated in the presence of 0.5 mM NAD^+, 50 mM Tris–HCl, 0.2 mM $CoCl_2$ at 46°C for 3 h. The reaction mixture was evaporated to give a crude DOI (68% yield by HPLC). The residue was subjected to the reductive dehydration with HI as above to give, after purification by prep. TLC, 2.5 mg of catechol (15% yield from G-6-P).

The above-mentioned chemo-enzymatic method involves much shorter reaction steps than those proposed by Frost et al.[2b] Although further improvement and modification appear to be necessary for large-scale operations, it should be emphasized here that the present approach has significant potential for the production of catechol and other benzenoids from widely available D-glucose and further implies significant opportunity for developing acceptable ‘green’ ways for the production of organochemical resources from sustainable agricultural and biomass products by utilizing enzymes involved in natural product biosynthesis.

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