Benzene-Free Synthesis of Hydroquinone

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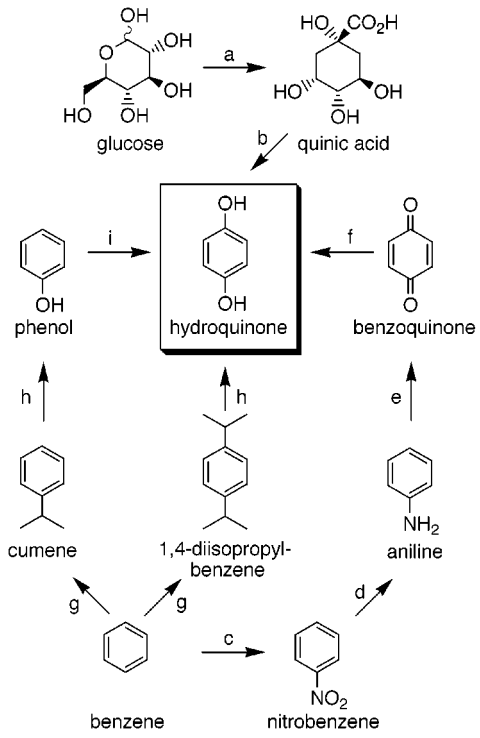
Abstract: All current routes for the synthesis of hydroquinone utilize benzene as the starting material. An alternate route to hydroquinone has now been elaborated from glucose. While benzene is a volatile carcinogen derived from nonrenewable fossil fuel feedstocks, glucose is nonvolatile, nontoxic, and derived from renewable plant polysacharrides. Glucose is first converted into quinic acid using microbial catalysis. Quinic acid is then chemically converted into hydroquinone. Under fermentor-controlled conditions, *Escherichia coli* QP1.1/pKD12.138 synthesizes 49 g/L of quinic acid from glucose in 20% (mol/mol) yield. Oxidative decarboxylation of quinic acid in clarified, decolorized, ammonium ion-free fermentation broth with NaOCl and subsequent dehydration of the intermediate 3(*R*),5(*R*)-trihydroxycyclohexanone afforded purified hydroquinone in 87% yield. Halide-free, oxidative decarboxylation of quinic acid in fermentation broth with stoichiometric quantities of $(NH_4)_2Ce(SO_4)_3$ and V_2O_5 afforded hydroquinone in 91% and 85% yield, respectively. Conditions suitable for oxidative decarboxylation of quinic acid with catalytic amounts of metal oxidant were also identified. Ag_3PO_4 at 2 mol % relative to quinic acid in fermentation broth catalyzed the formation of hydroquinone in 74% yield with $K_2S_2O_8$ serving as the cooxidant. Beyond establishing a fundamentally new route to an important chemical building block, oxidation of microbe-synthesized quinic acid provides an example of how the toxicity of aromatics toward microbes can be circumvented by interfacing chemical catalysis with biocatalysis.

Although chemistry has made significant strides in the synthesis of complex molecules laden with asymmetric centers and/or heteroatoms, establishing fundamentally new syntheses of structurally simple, building-block chemicals has attracted substantially less attention. A case in point is hydroquinone. As a pseudocommodity chemical synthesized globally at volumes of 4.5×10^7 kg/yr, hydroquinone is used as a photographic developer and as an intermediate in the synthesis of antioxidants and polymerization inhibitors.[1] Previously synthesized (Scheme 1) by a route employing stoichiometric amounts of MnO_2 to oxidize aniline, manufacture of hydroquinone is now dominated by Hock oxidation of 1,4-diisopropylbenzene and peroxide oxidation of phenol. Both Hock-type and peroxide oxidations constitute improvements in the synthesis of hydroquinone by virtue of reducing the number of required synthetic steps and eliminating byproduct salt streams. However, these improved syntheses of hydroquinone still have the common feature that volatile, carcinogenic benzene is the starting material. What are the options for synthesis of hydroquinone via a benzene-free route?

Woskresensky synthesized hydroquinone from quinic acid in 1838.[2] Although the source of quinic acid has historically been *Cinchona* bark,[3] a less exotic source of quinic acid was established in 1992 with the construction of a transgenic *Escherichia coli* strain that synthesized quinic acid from glucose under shake-flask conditions.[4] Oxidation of quinic acid with stoichiometric amounts of MnO_2 afforded low to modest yields of benzoquinone and hydroquinone.[4] Microbial synthesis of quinic acid and subsequent oxidation of this hydroaromatic established a benzene-free synthesis of hydroquinone from

Scheme 1[a]



[a] Conditions: (a) QP1.1/pKD12.138; (b) HOCl or $Ag_3PO_4/K_2S_2O_8$; (c) HNO_3, H_2SO_4; (d) Cu/SiO_2, H_2; (e) MnO_2, H_2SO_4; (f) Fe^0; (g) 2-propene, HZSM-12; (h) (i) O_2, NaOH, (ii) H_2SO_4; (i) HCO_2H, HCO_3H.

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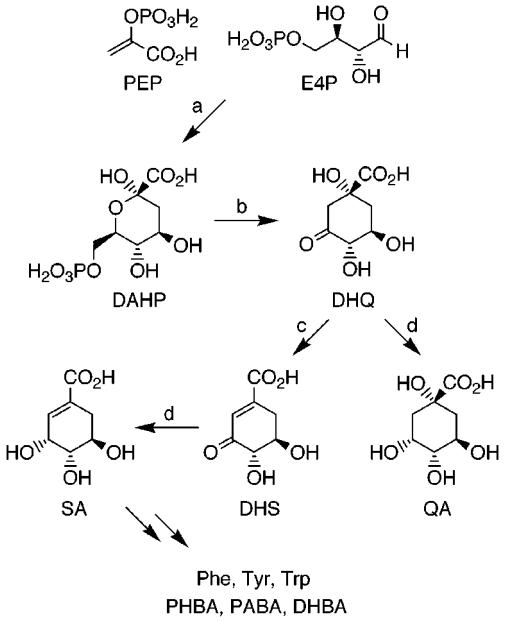
Scheme 2[a]



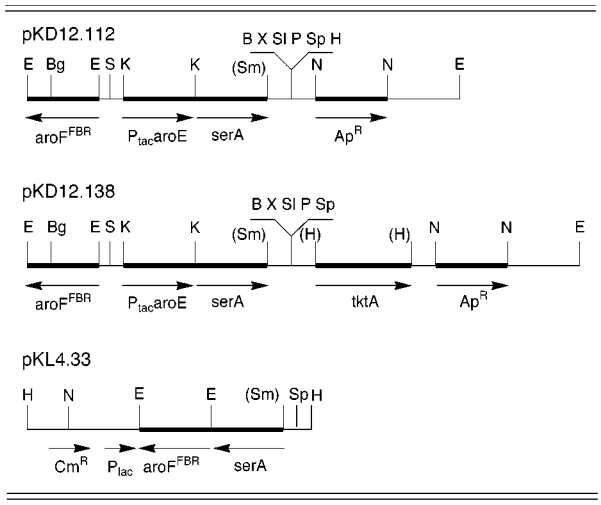
[a] Biosynthetic Intermediates (abbreviations): phosphoenolpyruvic acid (PEP), D-erythrose 4-phosphate (E4P), 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP), 3-dehydroquinic acid (DHQ), quinic acid (QA), 3-dehydroshikimic acid (DHS), shikimic acid (SA), L-phenylalanine (Phe), L-tyrosine (Tyr), L-tryptophan (Trp), *p*-hydroxybenzoic acid (PHBA), *p*-aminobenzoic acid (PABA), 2,3-dihydroxybenzoic acid (DHBA). Enzymes (encoding genes): (a) DAHP synthase (*aroF*^{FBR}); (b) 3-dehydroquinate synthase (*aroB*); (c) 3-dehydroquinate dehydratase (*aroD*); (d) shikimate dehydrogenase (*aroE*).

glucose (Scheme 1).[4] In this report, an *Escherichia coli* strain has been constructed that synthesizes substantially higher concentrations of quinic acid from glucose under fermentor-controlled conditions. High-yielding chemical methodology has also been elaborated for conversion of quinic acid in these fermentation broths into hydroquinone with stoichiometric amounts of NaOCl, $(NH_4)_2Ce(SO_4)_3$, or V_2O_5. Alternatively, catalytic quantities of Ag_3PO_4 can be used to convert quinic acid into hydroquinone with $K_2S_2O_8$ serving as a cooxidant.

Results

Construct Design and Culturing. The approach taken in this account to synthesize quinic acid from glucose had its genesis in the previously reported synthesis (Scheme 2) of shikimic acid from glucose.[5] Under certain culturing conditions, substantial concentrations of quinic acid were formed during microbial synthesis of shikimic acid.[5] This unwanted side reaction resulted from the unexpected ability of *aroE*-encoded shikimate dehydrogenase to reduce 3-dehydroquinic acid to quinic acid. Synthesis of shikimic acid from glucose was transformed into a route to quinic acid by employing an *E. coli* host strain lacking *aroD*-encoded 3-dehydroquinate dehydratase activity. Carbon flow directed into the common pathway of aromatic amino acid biosynthesis led to formation of 3-dehydroquinic acid. Rather than catalyzing the reduction of 3-dehydroshikimic acid in the microbial synthesis of shikimic acid, overexpressed, *aroE*-encoded shikimate dehydrogenase catalyzed the reduction of 3-dehydroquinic acid to afford product quinic acid (Scheme 2).

Table 1. Restriction Enzyme Maps of Plasmids[a]



[a] Restriction enzyme sites are abbreviated as follows: B = *Bam*HI, Bg = *Bgl*II, E = *Eco*RI, H = *Hin*dIII, K = *Kpn*I, N = *Nco*I, P = *Pst*I, S = *Sac*I, Sl = *Sal*I, Sm = *Sma*I, Sp = *Sph*I, X = *Xba*I. Parentheses indicate that the designated enzyme site has been eliminated. — vector DNA; ▬ insert DNA.

E. coli QP1.1 was constructed by the site-specific insertion of *aroB* into the *serA* locus of *E. coli* AB2848, which lacks 3-dehydroquinate dehydratase activity due to a mutation in its *aroD* locus. As a result of their inability to biosynthesize aromatic amino acids and vitamins, L-phenylalanine, L-tyrosine, L-tryptophan, *p*-hydroxybenzoic acid, *p*-aminobenzoic acid, and 2,3-dihydroxybenzoic acid were added to cultures of quinate-synthesizing *E. coli* QP1.1 constructs. Increasing the flow of carbon into the common pathway of aromatic amino acid biosynthesis was then accomplished with plasmid-localized *aroF*^{FBR},[6] which encodes a mutant isozyme of DAHP synthase insensitive to feedback inhibition by the aromatic supplements required for growth of *E. coli* QP1.1 constructs.

The two genomic *aroB* loci in *E. coli* QP1.1 increase the specific activity of 3-dehydroquinate synthase to a level where this enzyme is no longer an impediment to the flow of carbon through the common pathway of aromatic amino acid biosynthesis.[7] Disruption of the genomic *serA* locus attendant with insertion of the second *aroB* locus also provides the basis for plasmid maintenance. The *serA* locus encodes 3-phosphoglycerate dehydrogenase, which is an enzyme required for L-serine biosynthesis. Growth of *E. coli* QP1.1 in minimal salts medium unsupplemented with L-serine required maintenance and expression of plasmid-localized *serA*. Other genes localized on plasmids along with *serA* included the aforementioned *aroF*^{FBR} and *aroE*. Plasmids differed according to whether *tktA* was included (pKD12.138, Table 1) or not included (pKD12.112, Table 1) as an insert. Overexpression of *tktA*-encoded transketolase is hypothesized to increase the availability of D-erythrose 4-phosphate.[8] The availability of D-erythrose 4-phosphate apparently limits the in vivo activity of overexpressed DAHP synthase.[8]

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Table 2. Concentrations and Yields of Quinic Acid and 3-Dehydroquinic Acid Biosynthesized under Glucose-Limited Culture Conditions

	QA[c]	DHQ[c]	QA yield, %[d]	total yield, %[e]
QP1.1 pKD12.112[a]	40	2.3	15	16
QP1.1 pKD12.138[b]	49	3.3	20	21

[a] Cultured for 60 h. [b] Cultured for 48 h. [c] Units: g/L of quinic acid (QA), g/L of 3-dehydroquinic acid (DHQ). [d] (mol QA)/(mol glucose). [e] (mol QA + DHQ)/(mol glucose).

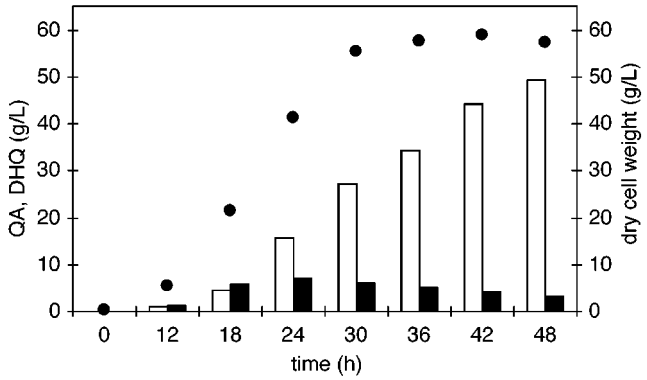

Figure 1. Biosynthesis of quinic acid under glucose-limited conditions by *E. coli* QP1.1/pKD12.138. Legend: quinic acid (QA, □), 3-dehydroquinic acid (DHQ, ■), dry cell weight (●).

E. coli QP1.1/pKD12.112 and *E. coli* QP1.1/pKD12.138 were cultured under fermentor-controlled conditions at 33 °C, pH 7.0, with dissolved oxygen maintained at a set point of 10% air saturation. Glucose addition was controlled by dissolved O_2 concentration with the rate of glucose addition dictated by a proportional-integral-derivative (PID) control loop. When dissolved oxygen levels exceeded the set point value indicating decreased microbial metabolism, the rate of glucose addition was increased and conversely the rate of glucose addition was decreased when dissolved oxygen levels declined below the set point value indicating increased microbial metabolism. A proportional gain (K_c) on the glucose PID control loop of 0.1 was used for culturing *E. coli* QP1.1/pKD12.112 and *E. coli* QP1.1/pKD12.138. These conditions maintained a steady-state concentration of glucose of approximately 0.2 mM.

E. coli QP1.1/pKD12.112 synthesized (Table 2) 40 g/L of quinic acid in 15% (mol/mol) yield in 60 h while *E. coli* QP1.1/pKD12.138 synthesized (Table 2, Figure 1) 49 g/L of quinic acid in 20% yield (mol/mol) in 48 h. The concentrations of hydroaromatics synthesized by QP1.1/pKD12.112 that are listed in Table 2 significantly differ from a previous report[5] and are the result of using crystalline quinic acid and 3-dehydroquinic acid standards to determine response factors for ^{1}H NMR analyses of culture supernatants. Amplified expression of transketolase clearly had an impact as reflected in the higher concentrations and yield of quinic acid synthesized from glucose by *E. coli* QP1.1/pKD12.138 relative to *E. coli* QP1.1/pKD12.112. Overexpression of transketolase also had a pronounced effect on the rate of quinic acid biosynthesis. Cultures of *E. coli* QP1.1/pKD12.138 were stopped after 48 h given the lack of significant increases in the concentration of quinic acid synthesized when *E. coli* QP1.1/pKD12.138 was cultured for longer (60 h) periods of time. By contrast, quinic acid biosynthesis in *E. coli* QP1.1/pKD12.112 required 60 h of cultivation before quinic acid synthesis leveled off.

The only metabolite other than quinic acid observed to accumulate to significant concentrations in the culture supernatants was 3-dehydroquinic acid. *E. coli* QP1.1/pKD12.112 synthesized (Table 2) 2.3 g/L of 3-dehydroquinic acid after 60 h while *E. coli* QP1.1/pKD12.138 synthesized (Table 2, Figure 1) 3.3 g/L of 3-dehydroquinic acid at 48 h. Concentrations of 3-dehydroquinic acid steadily increased in the culture supernatant of *E. coli* QP1.1/pKD12.138 reaching a maximum concentration of 7.1 g/L at 24 h (Figure 1). Concentrations of 3-dehydroquinic acid then steadily decreased between 24 and 48 h. This observation raises the intriguing possibility that some portion of the quinic acid synthesized by *E. coli* QP1.1/pKD12.138 was derived by transport back into the cytoplasm and subsequent reduction of 3-dehydroquinic acid that had been initially synthesized and exported into the culture supernatant.

Hydroquinone Toxicity. The toxicity of hydroquinone toward ethanologenic *E. coli* cultured on xylose under fermentative conditions has been analyzed from the perspective of hydroquinone's inhibition of sugar catabolism and damage to the plasma membrane.[25] To gauge the toxicity of hydroquinone toward *E. coli* cultured aerobically on glucose, *E. coli* QP1.1/pKL4.33 was used. Because significant amounts of quinic acid

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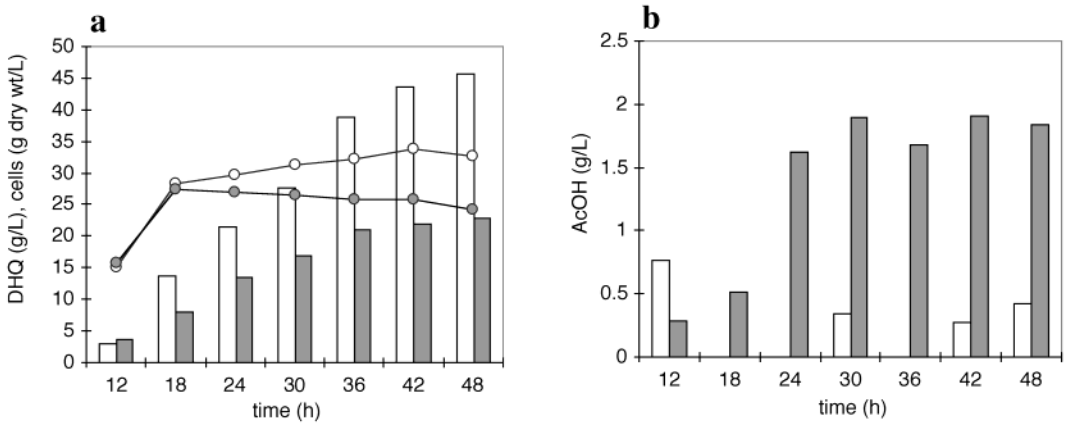


Figure 2. 3-Dehydroquinate-synthesizing *E. coli* QP1.1/pKL4.33. (a) 3-Dehydroquinic acid (DHQ) synthesis in the absence (□) versus presence (■) of added hydroquinone. Cell mass in the absence (○) versus the presence (●) of added hydroquinone. (b) Acetic acid (AcOH) formation in the absence (□) and presence (■) of added hydroquinone.

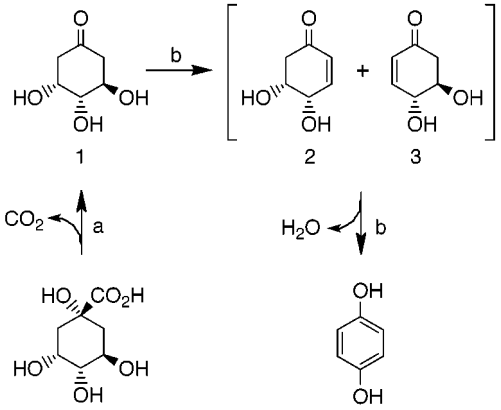
might be derived from initially exported 3-dehydroquinic acid, any changes in ratios of synthesized quinic and 3-dehydroquinic acids as a function of increasing hydroquinone concentration would complicate analysis of this aromatic's toxicity. This prompted use of *E. coli* QP1.1/pKL4.33, which synthesizes only 3-dehydroquinic acid, and differs from QP1.1/pKD12.112 only in the absence of the *aroE* insert encoding shikimate dehydrogenase. The fermentor conditions used to culture 3-dehydroquinate-synthesizing QP1.1/pKL4.33 were based on the parameters used to culture quinate-synthesizing QP1.1/pKD12.112 and QP1.1/pKD12.138. A sterile, aqueous solution of hydroquinone was added to the fermentor run to a final concentration of 2 g/L at 12 h.

QP1.1/pKL4.33 was able to grow and synthesize 3-dehydroquinic acid in the presence of added hydroquinone. However, 3-dehydroquinic acid synthesis dropped by approximately 50% upon addition of hydroquinone (Figure 2a). Less cell mass was also formed (Figure 2a) and increased amounts of acetic acid were produced (Figure 2b) in the presence of added hydroquinone. The specific activity of DAHP synthase was the only parameter examined that did not significantly change over the course of the fermentor run when hydroquinone was added. As the first enzyme in the common pathway, the specific activity of DAHP synthase significantly determines carbon flow directed into synthesis of 3-dehydroquinic acid.[9] The reduced concentration of synthesized 3-dehydroquinic acid, reduction in cell mass, and increased production of acetic acid observed for QP1.1/pKL4.33 indicate that hydroquinone concentrations as low as 2 g/L are toxic to microbial growth and metabolism.

Hypochlorite Oxidations. Chemical conversion of quinic acid into hydroquinone (Scheme 3) required the development of reaction methodology appropriate for use in aqueous medium and compatible for use in the complex solution matrix associated with fermentation broths. Cells were removed by centrifugation. Heating this culture supernatant to reflux followed by acidification resulted in precipitation of proteins, which were removed by centrifugation. The clarified fermentor broth was then decolorized with activated charcoal. Clarified, decolorized fermentation broth was then passed through a strong cation-exchange resin (Dowex 50 H^+) to remove ammonium ion. Ammonium hydroxide along with H_2SO_4 was used during the course of the fermentation runs to maintain the culture medium at pH 7. Hypochlorite oxidation of quinic acid was not observed in lieu of ammonium ion removal.

Sodium hypochlorite was added to clarified, decolorized, ammonium ion-free fermentation broth. The reaction solution

Scheme 3[a]



[a] Conditions: (a) (i) NaOCl, room temperature, (ii) 2-propanol, room temperature; (b) reflux.

was then acidified and reacted at room temperature to give 3(*R*),5(*R*)-trihydroxycyclohexanone (**1**, Scheme 3) based on ^{1}H NMR analysis of the crude reaction solution. Excess hypochlorite was then quenched with 2-propanol. Without purification, the resulting solution was refluxed under an inert atmosphere for 10 h. The concentration of cyclohexanone **1** decreased, the concentration of hydroquinone increased, and α,β-unsaturated enones **2** and **3** accumulated as intermediates (Scheme 3, Figure 3). Identification and quantification of enone intermediates was accomplished by ^{1}H NMR analysis of aliquots withdrawn from the reaction solution and comparison with the ^{1}H NMR spectra of enones **2** and **3** independently synthesized from 3(*R*),5(*R*)-trihydroxycyclohexanone (**1**) and butane 2,3-bisacetal-protected methyl quinate, respectively. Extraction of the dehydration/aromatization reaction solution with *tert*-butyl methyl ether followed by sublimation afforded purified hydroquinone in 87% overall yield from quinic acid.

Chloride-Free Oxidations. Quinic acid was also converted into 3(*R*),5(*R*)-trihydroxycyclohexanone (**1**, Scheme 3) by using electrochemical oxidation. The electrolysis was performed at room temperature in a 50 cm^3 electrolysis cell fitted with a pair of Pt electrodes (2 × 1.35 cm^2). Culture supernatant containing quinic acid was adjusted to pH 10 by addition of 1 N aqueous NaOH prior to electrolysis. Electrolysis at a current density of 400 mA/cm^2 for 4 h afforded 3(*R*),5(*R*)-trihydroxycyclohexanone (**1**, Scheme 3) in 24% yield along with an 8% yield of formic acid and a 25% yield of unreacted quinic acid. Electrolysis at a higher current density (600 mA/cm^2) or

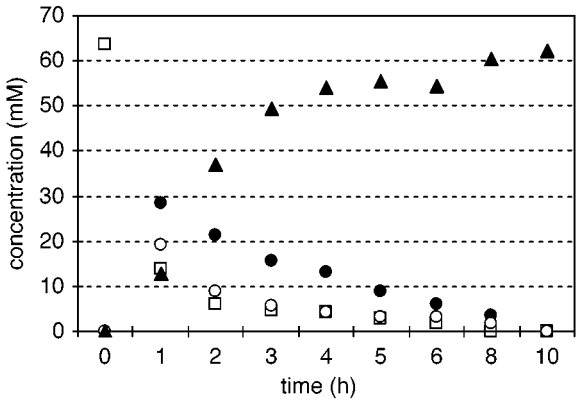


Figure 3. Dehydration of 3(*R*),5(*R*)-trihydroxycyclohexanone (**1**) (□) to hydroquinone (▲) via intermediacy of 4(*S*),5(*R*)-dihydroxy-2-cyclohexen-1-one (**2**) (●) and 4(*R*),5(*R*)-dihydroxy-2-cyclohexen-1-one (**3**) (○).

Table 3. Reaction Conditions and Crude Product Yields for Chloride-Free Oxidation of Quinic Acid

entry	oxidant/ catalyst (equiv)[a]	conditions	hydroquinone yield,[b] %
1	$(NH_4)_2Ce(SO_4)_3$ (2.4)	room temperature, 30 min; reflux, 10 h	91
2	V_2O_5 (1.1)	50 °C, 4 h; reflux, 8 h	85
3	$K_2S_2O_8$ (1.2)/Ag_3PO_4 (0.10)	50 °C, 4 h; reflux, 8 h	85
4	$K_2S_2O_8$ (1.2)/Ag_3PO_4 (0.02)	50 °C, 4 h; reflux, 8 h	74
5	$K_2S_2O_8$ (1.2)/Ag_3PO_4 (0.01)	50 °C, 4 h; reflux, 8 h	51

[a] Units: mol oxidant/mol quinic acid. [b] Reported yields are based on isolated, purified hydroquinone.

electrolysis for a longer period of time resulted in increased formation of formic acid without increased yields of ketone **1**.

Transition metal-mediated oxidative decarboxylation of quinic acid used stoichiometric amounts of Ce^{4+} and V^{5+} salts (Table 3). Oxidation of quinic acid in clarified, decolorized, ammonium ion-free fermentation broth at room temperature with ceric ammonium sulfate ($(NH_4)_2Ce(SO_4)_3$) followed by refluxing the reaction solution afforded hydroquinone in 91% isolated yield (entry 1, Table 3). Addition of vanadium pentoxide (V_2O_5) and H_2SO_4 to quinate-containing fermentation broth, which had been clarified, decolorized, and made ammonium ion-free, led to an 85% isolated yield of hydroquinone (entry 2, Table 3) after heating the reaction solution to 50 °C and final reaction at reflux. The oxidations and associated reaction conditions summarized in Table 3 are noteworthy in the absence of benzoquinone formation resulting from oxidation of the initially formed hydroquinone.

In route to identification of metals that could be used in catalytic amounts, CuCl with O_2 as a cooxidant and $FeSO_4$ or $RuCl_3$ with H_2O_2 as the cooxidant were examined.[10] No oxidative decarboxylation of quinic acid in clarified, decolorized, ammonium ion-free fermentation broth was observed. However, use of substoichiometric, catalytic amounts of Ag_3PO_4 along with potassium persulfate ($K_2S_2O_8$) as the cooxidant did lead to oxidative decarboxylation. For these Ag_3PO_4-catalyzed oxidations, removal of ammonium ions was critical. The presence of ammonium ions led to formation of a silver mirror and an absence of hydroquinone formation. Oxidation of quinic acid in partially purified culture supernatant with a catalytic amount of Ag_3PO_4 (10 mol %) and cooxidant $K_2S_2O_8$ heated initially at 50 °C followed by heating of the reaction solution at reflux afforded an 85% yield of hydroquinone (entry 3, Table 3). Quantities of Ag_3PO_4 catalyst as low as 2 mol % and 1 mol % relative to quinic acid could be used (entries 4 and 5, Table 3) although significant decreases in the yields of hydroquinone were observed.

Discussion

Chemical Synthesis of Hydroquinone. Oxidation of aniline (Scheme 1) is the oldest route for synthesis of hydroquinone and still accounts for about 10% of global hydroquinone manufacture.[1] Aniline is oxidized by MnO_2 in aqueous H_2SO_4 to form benzoquinone. Reduction of benzoquinone with Fe^0 or hydrogenation then affords product hydroquinone. Synthesis of hydroquinone via intermediacy of nitrobenzene and aniline generates large quantities of $MnSO_4$, $(NH_4)_2SO_4$, and iron oxide salts.[1]

Hock oxidation of 1,4-diisopropylbenzene (Scheme 1) accounts for approximately 60% of global hydroquinone production. The 1,4-diisopropylbenzene is synthesized by Freidel−Crafts reaction of benzene or cumene with propylene or 2-propanol. Catalyzed air oxidation of 1,4-diisopropylbenzene produces dihydroperoxide, hydroxyhydroperoxide, and dicarbinol products. The hydroxyhydroperoxide and dicarbinol are converted into the dihydroperoxide upon treatment with H_2O_2. Acid-catalyzed cleavage of the dihydroperoxide produces acetone and hydroquinone. During the acid-catalyzed cleavage, explosive acetone hydroperoxides are formed.[1]

Reaction of phenol with H_2O_2 in the presence of acid catalysts leads to a mixture of hydroquinone and catechol (Scheme 1). Homogeneous acid catalysis employs both organic and inorganic acids such as formic, sulfuric, and trifluoromethanesulfonic acids. Heterogeneous acid catalysis is also possible by using a variety of different materials including synthetic zeolites. The acid catalyst employed during reaction of phenol with H_2O_2 significantly controls the ratio of hydroquinone to catechol.[1] Hydroxylation of phenol accounts for approximately 30% of global hydroquinone synthesis.

Microbial Synthesis of Hydroquinone. Microbe-catalyzed routes to hydroquinone have included the butane-catabolizing bacterium *Mycobacterium* sp. HB50, which synthesized hydroquinone from phenol, and the methane-catabolizing *Methylosinus trichosporium* OB3b, which catalyzed the conversion of benzene into hydroquinone.[11] A continuous reaction system was developed for *Mycobacterium* sp. HB50 allowing phenol to be converted into hydroquinone with a specific volumetric productivity of 2−3 g/L/h.[11a] *Methylosinus trichosporium* OB3b synthesized low concentrations (approximately 1 g/L) of hydroquinone from benzene with use of a chemostat to control reaction conditions.[11b] Phenol, hydroquinone, and benzoquinone are all toxic toward microbes.[11a] A key advantage cited for use of *Mycobacterium* sp. was its greater resilience toward hydroquinone and benzoquinone toxicity relative to *Methylosinus trichosporium*.[11a] Even with this enhanced resistance, the *Mycobacterium* sp. HB50 strain used for conversion of phenol into hydroquinone was derived by random chemical mutagenesis of a parent *Mycobacterium* strain by selection for resistance to hydroquinone.[11a]

In theory, glucose can be converted into hydroquinone via a completely biocatalytic route involving intermediacy of *p*-hydroxybenzoic acid. Glucose has been converted into *p*-hydroxybenzoic acid in modest yields by using recombinant *Escherichia coli* constructs.[12] Conversion of this *p*-hydroxybenzoic acid into hydroquinone is a catalytic activity associated with *p*-hydroxybenzoate 1-hydroxylase, an enzyme found in *Candida parapsilosis*.[13] To construct a single microbe capable

of catalyzing the conversion of glucose into hydroquinone, the *C. parapsilosis* gene encoding *p*-hydroxybenzoate 1-hydroxylase would likely have to be isolated and then expressed in a *p*-hydroxybenzoate-synthesizing microbial host. The toxicity of intermediate *p*-hydroxybenzoic acid is also problematic. Although not possessing the antimicrobial activity of parabens, unesterified *p*-hydroxybenzoic acid is still toxic toward microbes.[12b]

Conversion of phenol to hydroquinone catalyzed by *Mycobacterium* sp. HB50 and conversion of benzene to hydroquinone catalyzed by *Methylosinus trichosporium* OB3b share with the aforementioned (Scheme 1) chemical routes the feature of directly or indirectly using fossil fuel-derived benzene as the starting material. Microbial conversion of glucose to hydroquinone via intermediacy of *p*-hydroxybenzoic acid, although avoiding use of an aromatic starting material, still must contend with the microbial toxicity of an aromatic intermediate. Hydroquinone is also toxic toward *E. coli* as indicated by its impact on 3-dehydroquinate-synthesizing QP1.1/pKL4.33 (Figure 2). However, the key distinction between the chemical oxidation of microbe-synthesized quinic acid as a route to hydroquinone relative to all reported or theoretical biocatalytic syntheses of hydroquinone is that the microbial catalyst never comes into contact with a toxic aromatic starting material, aromatic intermediate, or aromatic product. Quinic acid at neutral pH does not adversely effect either growth or metabolism of *E. coli*.

Microbial Synthesis of Quinic Acid. In the previously reported microbe-catalyzed synthesis of quinic acid from glucose, 3-dehydroquinic acid was reduced to quinic acid in *E. coli* AB2848/pKD136/pTW8090A by heterologous expression of *qad*-encoded quinate dehydrogenase isolated from *Klebsiella pneumoniae*.[4] *E. coli* QP1.1/pKD12.112 and QP1.1/pKD12.138 used *aroE*-encoded shikimate dehydrogenase isolated from *E. coli*. This meant that promoter compatibility and codon usage were completely avoided as factors requiring consideration in route to achieving adequate overexpression of the enzyme that reduced 3-dehydroquinic acid to quinic acid.

E. coli AB2848/pKD136/pTW8090A was cultured under shake-flask conditions and required the presence of antibiotics in its culture medium for stable maintenance of the two plasmids. The quinate-synthesizing constructs used in this study, *E. coli* QP1.1/pKD12.112 and QP1.1/pKD12.138, relied on nutritional pressure as opposed to resistance to antibiotics for plasmid maintenance. In our experience, *E. coli* constructs that employ nutritional pressure for maintenance of a single plasmid are quite amenable to culturing under fermentor conditions. This is an important consideration given that it is generally difficult to control oxygenation and pH during growth of microbes in shake flasks. Microbes cultured under shake-flask conditions are also under physiological stress due to the glucose-rich environment present immediately after inoculation that changes to a glucose-limited environment as the cultures grow. Fermentor-controlled cultures allow oxygenation, pH, and glucose availability to be maintained at set, constant values over the entire course of microbial growth and synthesis of quinic acid.

While the previously reported *E. coli* AB2848/pKD136/pTW8090A synthesized only 4.8 g/L of quinic acid,[4] *E. coli* QP1.1/pKD12.138 synthesized 49 g/L of quinic acid. The conversion of glucose (80 mM) into quinic acid (20 mM) catalyzed by *E. coli* AB2848/pKD136/pTW8090A suggests an apparent yield for synthesis of quinic acid from glucose that is higher than the 20% yield achieved using *E. coli* QP1.1/pKD12.138. However, this comparison is not meaningful since *E. coli* AB2848/pKD136/pTW8090A was first cultured in rich medium, harvested, and then resuspended in minimal salts medium where synthesis of quinic acid occurred. The yield (Table 3) of quinic acid synthesized by *E. coli* QP1.1/pKD12.112 and QP1.1/pKD12.138 reflects both the amount of glucose consumed to form biomass as well as the amount of glucose consumed to synthesize quinic acid. Nonetheless, significant improvement in the yield of quinic acid microbially synthesized from glucose remains to be achieved. The theoretical maximum yield for synthesis of quinic acid from glucose with *E. coli* is 43% (mol/mol).[6a] In addition, a variant of *E. coli* QP1.1/pKD12.138 will ultimately need to be constructed that does not require addition of aromatic amino acids and vitamins. Cultures of *E. coli* AB2848/pKD136/pTW8090A, QP1.1/pKD12.112, and QP1.1/pKD12.138 needed to be supplemented with aromatic amino acids and aromatic vitamins because of the mutation rendering 3-dehydroquinate dehydratase catalytically inactive.

Conversion of Quinic Acid to Hydroquinone. Reaction methodology previously employed for the chemical conversion of quinic acid to hydroquinone (Scheme 2) required stoichiometric amounts of MnO_2.[4] Purified quinic acid in refluxing aqueous solution was converted by MnO_2 into hydroquinone in 10% yield.[4] Although a 70% yield was realized when purified quinic acid was reacted in acidified aqueous solutions with MnO_2, the product was benzoquione and not hydroquinone.[4] A lower 40% yield of benzoquinone was realized upon reaction of quinic acid in acidified fermentation broth heated to reflux with MnO_2.[4]

The stoichiometric reaction of quinic acid with MnO_2 was problematic from the standpoint of the byproduct salt stream. This is the same problem associated with the commercial route to hydroquinone where stoichiometric amounts of MnO_2 are used to oxidize aniline (Scheme 1).[1] With the improvements achieved in the biocatalytic methodology for conversion of glucose into quinic acid, attention was focused on improving the companion chemical methodology for conversion of quinic acid into hydroquinone. Reagents were needed that were sufficiently robust for use in fermentation broths while being mild enough to avoid overoxidation of hydroquinone to benzoquinone.

Oxidative decarboxylation of quinic acid to afford 3(*R*),5(*R*)-trihydroxycyclohexanone (**1**, Scheme 3) conforms to previously reported reactions of hypochlorite with α-hydroxycarboxylic acids.[14] The stability of 3(*R*),5(*R*)-trihydroxycyclohexanone (**1**) under the oxidative decarboxylation conditions is an important factor for high-yielding conversion of quinic acid into hydroquinone. Dehydration and aromatization of ketone **1** to hydroquinone during oxidative decarboxylation of quinic acid would likely result in overoxidation of hydroquinone to benzoquinone or chlorination of hydroquinone.

In route to identifying a chlorine-free oxidative decarboxylation of quinic acid, electrochemical oxidation of quinic acid was explored. Electrochemical oxidation of α-hydroxycarboxylic acids has been reported.[15] However, only approximately 50% of the mass balance could be accounted for after partial electrochemical oxidation of quinic acid. The lack of improved 3(*R*),5(*R*)-trihydroxycyclohexanone (**1**) yields and increased formation of formic acid when quinic acid was electrochemically oxidized for longer time increments led to the examination of oxidative decarboxylation of quinic acid with Ce^{4+} and V^{5+}. Oxidative decarboxylation of α-hydroxycarboxylic acids with Ce^{4+} and V^{+5} has been reported[16] and suggests that metal-mediated oxidation of the α-hydroxycarboxylate quinic acid

might be a substantial improvement over electrochemical oxidation. This anticipation was borne out by the favorable yields (Table 3) of hydroquinone formed with $(NH_4)_2Ce(SO_4)_3$ and V_2O_5 as oxidants.

Moving from use of stoichiometric to catalytic amounts of metal for oxidation of quinic acid took advantage of the reported ability of Ag^{1+} to accelerate the oxidation of carboxylic acids by peroxydisulfate ion.[17] Apparently, these oxidative decarboxylations are catalyzed by a Ag^{2+} species formed from peroxydisulfate oxidation of Ag^{1+}.[17] Use of inorganic phosphate as the counteranion for Ag^{1+} followed from inorganic phosphate being the dominant oxyanion present in fermentation broth. Overoxidation of hydroquinone to benzoquinone was likely prevented by the use of only a small molar excess of $K_2S_2O_8$ relative to quinic acid.

Environmental, Health, and Carbon Management Considerations. Exposure to benzene, which has been linked to both acute myeloid leukemia and non-Hodgkin's lymphoma,[18] continues to create challenges to the chemical industry. With annual production of benzene in the U.S. at approximately 8×10^9 kg,[19] high costs have been cited by the U.S. chemical industry to be a major impediment to reducing exposure limits for benzene.[20] Ultimately, the most effective way of dealing with benzene's human health risk may be to circumvent its use. Such a solution, although conceptually simple, requires the elaboration of fundamentally new syntheses for aromatic chemicals and products derived from aromatics. The synthesis of hydroquinone via chemical oxidation of microbe-synthesized quinic acid can be viewed as being part of this process. Catechol, adipic acid, and phenol are examples of other larger volume chemicals currently synthesized from benzene where syntheses have been successfully elaborated from glucose.[21]

There are also economic opportunity costs associated with continued reliance on benzene as a chemical building block. As declining reserves and production lead to continuing increases in the price of petroleum,[22] the costs of using petroleum-derived benzene will likewise increase. The availability of glucose derived from corn starch, by contrast, continues to increase. From 1950 to 2000, the yield per acre of corn in the U.S. increased at an annual rate of 2−4%.[23] The starch component of the U.S. corn harvest in 2000 was 143×10^9 kg. Continued increases in the yield per acre of corn starch and development of corn fiber and lignocellulose as carbohydrate feedstocks suggest that glucose and other carbohydrates will become an increasingly attractive source of carbon for synthesis of chemicals. Sequestration of CO_2 with the synthesis of hydroquinone is another consideration given that glucose is essentially an immobilized form of CO_2. As the world moves to national CO_2 budgets set by international treaty, chemicals synthesized from carbohydrates such as glucose may be important CO_2 credits.

QP1.1/pKD12.138 synthesizes 10-fold higher concentrations of quinic acid from glucose than previously reported in the literature.[4] The fermentor-controlled conditions for this conversion that are possible with QP1.1/pKD12.138, as opposed to the previously employed shake-flask conditions, are amenable to scale-up for evaluation of larger volume cultivation. Oxidation of quinic acid with NaOCl or $Ag_3PO_4/K_2S_2O_8$ allows quinic acid to be converted into hydroquinone in high yield and without overoxidation to benzoquinone. This contrasts with the previous report[4] of quinic acid oxidation, which either afforded hydroquinone in low yield or required reduction of initially formed benzoquinone. Synthesis of hydroquinone from glucose clearly has aspects that need to be further optimized or modified before this route can be used on an industrial scale. However, the chemical oxidation of microbially synthesized quinic acid described in this report moves the synthesis of hydroquinone from glucose from a proof-of-concept conversion to a route with prospects for supplanting currently employed syntheses of hydroquinone where benzene serves as the starting material.

Experimental Section

General Chemistry. ^{1}H NMR spectra were recorded at 300 MHz on a Varian Gemini-300 spectrometer. Chemical shifts for ^{1}H NMR spectra are reported (in parts per million) relative to internal tetramethylsilane (Me_4Si, δ 0.0 pm) with $CDCl_3$ as a solvent, and to sodium 3-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$ (TSP, δ 0.0 ppm) when D_2O was the solvent. ^{13}C NMR spectra were recorded at 75 MHz on a Gemini-300 spectrometer. Chemical shifts for ^{13}C NMR spectra are reported (in parts per million) relative to $CDCl_3$ (δ 77.0 ppm) or internal acetonitrile (CH_3CN, δ 3.69 ppm) in D_2O. To determine yields of hydroquinone in crude reaction solutions (Table 3), a portion (0.5−1.0 mL) of the solution was concentrated to dryness, concentrated to dryness one additional time from D_2O, and then redissolved in 1 mL of D_2O containing 10 mM TSP. The concentration of hydroquinone in the solution was determined by the ratio of the integrated ^{1}H NMR resonance at δ 6.82 with the integrated resonance corresponding to TSP at δ 0.00.

FAB mass spectra were obtained on a double-focusing mass spectrometer. Elemental analyses were performed by Atlantic Microlab Inc. (Norcross, GA). Silica gel 60 (40−63 μm, E. Merck) was used for flash chromatography. Analytical thin-layer chromatography (TLC) utilized precoated plates of silica gel 60A (0.25 mm, Whatman). TLC plates were visualized by immersion in 7% phosphomolybdic acid in ethanol followed by heating. Pyridine, Et_3N, and CH_2Cl_2 were distilled from calcium hydride under nitrogen. Tetrahydrofuran was distilled under nitrogen from sodium benzophenone ketyl. Methanol was dried over activated 3A molecular sieves. Organic solutions of products were dried over $MgSO_4$. Ag_3PO_4 was purchased from Aldrich.

Culture Medium. All solutions were prepared in distilled, deionized water. LB medium[24] contained (1 L) Bacto tryptone (10 g), Bacto yeast extract (5 g), and NaCl (10 g). M9 salts (1 L) contained Na_2HPO_4 (6 g), KH_2PO_4 (3 g), NH_4Cl (1 g), and NaCl (0.5 g). M9 minimal medium contained D-glucose (10 g), $MgSO_4$ (0.12 g), and thiamine hydrochloride (0.001 g) in 1 L of M9 salts. M9 medium (1 L) was supplemented where appropriate with L-phenylalanine (0.040 g), L-tyrosine (0.040 g), L-tryptophan (0.040 g), *p*-hydroxybenzoic acid (0.010 g), potassium *p*-aminobenzoate (0.010 g), and 2,3-dihydroxybenzoic acid (0.010 g). Antibiotics were added where indicated to the following final concentrations: ampicillin (Ap), 50 μg/mL; and chloramphenicol (Cm), 20 μg/mL. Solutions of M9 salts, $MgSO_4$, and glucose were autoclaved individually and then mixed. Solutions of amino acids, aromatic vitamins, thiamine hydrochloride, and antibiotics were sterilized through 0.22-μm membranes. Solid medium was prepared by addition of Difco agar to a final concentration of 1.5% (w/v) to the liquid medium.

The standard fermentation medium (1 L) contained K_2HPO_4 (7.5 g), ammonium iron(III) citrate (0.3 g), citric acid monohydrate (2.1 g), L-phenylalanine (0.7 g), L-tyrosine (0.7 g), L-tryptophan (0.35 g), and concentrated H_2SO_4 (1.2 mL). Fermentation medium was adjusted to pH 7.0 by addition of concentrated NH_4OH before autoclaving. The following supplements were added immediately prior to initiation of the fermentation: glucose, $MgSO_4$ (0.24 g), *p*-hydroxybenzoic acid (0.010 g), potassium *p*-aminobenzoate (0.010 g), 2,3-dihydroxybenzoic acid (0.010 g), and trace minerals including $(NH_4)_6(Mo_7O_{24})\cdot 4H_2O$ (0.0037 g), $ZnSO_4\cdot 7H_2O$ (0.0029 g), H_3BO_3 (0.0247 g), $CuSO_4\cdot 5H_2O$ (0.0025 g), and $MnCl_2\cdot 4H_2O$ (0.0158 g). Glucose and $MgSO_4$ (1 M) solutions were autoclaved separately while solutions of aromatic vitamins and trace minerals were sterilized through 0.22-μm membranes. Antifoam (Sigma 204) was added as needed.

Fed-Batch Fermentations. Fermentations employed a 2.0 L working capacity B. Braun M2 culture vessel. Utilities were supplied by a B. Braun Biostat MD controlled by a DCU-1. Data acquisition utilized a Dell Optiplex Gs$^+$ 5166M personal computer (PC) equipped with B. Braun MFCS/Win software (v1.1). Temperature, pH, and glucose feeding were controlled with PID control loops. Temperature was

maintained at 33 °C for all fermentations. pH was maintained at 7.0 by addition of concentrated NH_4OH or 2 N H_2SO_4. Dissolved oxygen (D.O.) was measured with use of a Mettler-Toledo 12 mm sterilizable O_2 sensor fitted with an Ingold A-type O_2 permeable membrane. D.O. was maintained at 10% air saturation. The initial glucose concentration in the fermentation medium ranged from 20 to 28 g/L.

Inoculants were started by introduction of a single colony picked from an agar plate into 5 mL of M9 medium. Ampicillin was added to the medium at each step of the inoculant preparation. Cultures were grown at 37 °C with agitation at 250 rpm until they were turbid (18−24 h) and subsequently transferred to 100 mL of M9 medium. Cultures were grown at 37 °C and 250 rpm for an additional 8 to 10 h. After the culture reached an appropriate OD_{600} (3.0−3.5), the inoculant was transferred into the fermentation vessel and the batch fermentation was initiated ($t = 0$ h).

Three staged methods were used to maintain D.O. concentrations at 10% air saturation during the fermentations. With the airflow at an initial setting of 0.06 L/L/min, the D.O. concentration was maintained by increasing the impeller speed from its initial set point of 50 rpm to its preset maximum of 940 rpm. With the impeller rate constant at 940 rpm, the mass flow controller then maintained the D.O. concentration by increasing the airflow rate from 0.06 L/L/min to a preset maximum of 1.0 L/L/min. At constant impeller speed and constant airflow rate, the D.O. concentration was finally maintained at 10% air saturation for the remainder of the fermentation by oxygen sensor-controlled glucose feeding. At the beginning of this stage, the D.O. concentration fell below 10% air saturation due to residual initial glucose in the medium. This lasted for approximately 0.5 to 1 h before glucose (65% w/v) feeding commenced. The glucose feed PID control parameters were set to 0.0 s (off) for the derivative control (τ_D) and 999.9 s (minimum control action) for the integral control (τ_I). X_p was set to 950% to achieve a K_c of 0.1.

Analysis of Fermentation Broth. Samples (5−10 mL) of fermentation broths were taken at the indicated timed intervals. Cell densities were determined by dilution of fermentation broth with water (1:100 or 1:200) followed by measurement of absorption at 600 nm (OD_{600}). Dry cell weight (g/L) was calculated by using a conversion coefficient of 0.43 g/L/OD_{600}. The remaining fermentation broth was centrifuged to obtain cell-free broth.

Solute concentrations in the cell-free broth were quantified by ^{1}H NMR. Solutions were concentrated to dryness under reduced pressure, concentrated to dryness one additional time from D_2O, and then redissolved in D_2O containing a known concentration of the sodium salt of TSP. Concentrations were determined by comparison of integrals corresponding to each compound with the integral corresponding to TSP (δ 0.00 ppm). Response factors were based on standard concentration curves with use of crystalline quinic and 3-dehydroquinic acids. Compounds were quantified by using response factors based on the following resonances: quinic acid (δ 4.16, m, 1 H) and 3-dehydroquinic acid (δ 4.28, d, 1 H).

Fermentation Broth Workup. Following removal of cells by centrifugation of fermentor broth at 4200*g* for 15 min, 1 L of crude fermentation broth containing quinic acid was refluxed for 1 h. After the solution was cooled to room temperature, concentrated sulfuric acid was added to a final pH of 2.5. Precipitated proteins were removed by centrifugation at 17000*g* for 20 min. This clarified solution was then stirred with charcoal (Darco KB-B, 20 g) at room temperature for 2 h. After filtration, the decolorized solution was passed through a Dowex 50 (H^+) column at 4 °C to afford clarified, decolorized, ammonium ion-free fermentation broth with a 98% recovery of quinic acid.

Synthesis of Hydroquinone by Hypochlorite Oxidation of Quinic Acid. To 1.12 L of clarified, decolorized, ammonium ion-free fermentation broth containing quinic acid (53.5 g, 0.279 mol) in a 5 L three-neck round-bottom flask at room temperature were simultaneously added commercial bleach (Clorox, 5.25% NaOCl, 1.27 mol) and H_2SO_4 (0.34 mol) with stirring over a 1 h period. The mixture was stirred for an additional 2 h. 2-Propanol (130 mL, 1.70 mol) was added to quench the unreacted hypochlorite. Without further purification, the resulting solution containing 3(*R*),5(*R*)-trihydroxycyclohexanone was heated to reflux under Ar for 10 h. After the solution was cooled to room temperature, hydroquinone was extracted into *tert*-butyl methyl ether (4 × 500 mL) and the combined organic phase was dried over anhydrous $MgSO_4$. The solution was stirred with charcoal (20 g) for 10 min and then filtered through a pad of Celite. The Celite was washed with additional *tert*-butyl methyl ether (200 mL). The filtrates were then concentrated in vacuo to obtain hydroquinone as a brown solid (29.4 g, 94%). Sublimation of the isolated materials yielded hydroquinone (27.3 g, 87%) as a white solid. The ^{1}H NMR and ^{13}C NMR spectra were identical with those of authentic hydroquinone.

Ag_3PO_4-Catalyzed Oxidation of Quinic Acid. To a clarified, decolorized, ammonium ion-free fermentation broth containing quinic acid (0.960 g, 5.00 mmol) was added $K_2S_2O_8$ (1.62 g, 6.00 mmol) and Ag_3PO_4 (0.0481 g, 0.115 mmol). The heterogeneous solution was stirred at 50 °C for 4 h and then refluxed for 8 h under Ar. After filtration of the crude reaction solution and extraction with EtOAc (3 × 50 mL), the organic layer was dried and concentrated. Purification of the residue by flash chromatography (1:1 EtOAc/hexane, v/v) gave hydroquinone (0.407 g, 74%) as a white solid. The ^{1}H NMR and ^{13}C NMR spectra were identical with those of authentic hydroquinone.

Oxidation of Quinic Acid by $(NH_4)_4Ce(SO_4)_4$. Ceric ammonium sulfate (7.15 g, 12.0 mmol) was added over a 30 min period to a clarified, decolorized, ammonium ion-free fermentation broth containing quinic acid (0.960 g, 5.00 mmol) at pH 1.5. The solution was then refluxed for 10 h under an Ar atmosphere. After the mixture was cooled to room temperature, the resulting solution was filtered to obtain an aqueous solution of hydroquinone, which was then extracted with EtOAc (3 × 50 mL). The combined organic layers were dried and concentrated to a brown solid, which was purified by flash chromatography (1:1 EtOAc/hexane, v/v) to afford hydroquinone (0.503 g, 91%) as a white solid. The ^{1}H NMR and ^{13}C NMR spectra were identical with those of authentic hydroquinone.

Oxidation of Quinic Acid by V_2O_5. To a clarified, decolorized, ammonium ion-free fermentation broth containing quinic acid (0.960 g, 5.00 mmol) at pH 1.5 were added V_2O_5 (1.00 g, 5.50 mmol) and H_2SO_4 (7.5 mmol). The solution was stirred at 50 °C for 4 h and then heated to reflux for 8 h under an Ar atmosphere. After extraction of the crude reaction solution with EtOAc (3 × 50 mL), the organic layer was dried and concentrated to dryness. Purification by flash chromatography (1:1 EtOAc/hexane, v/v) afforded hydroquinone (0.466 g, 85%) as a white solid. The ^{1}H NMR and ^{13}C NMR spectra were identical with those of authentic hydroquinone.

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Supporting Information Available: Experimental details for genetic manipulations and synthesis of 3(*R*),5(*R*)-trihydroxycyclohexanone (**1**), 4(*S*),5(*R*)-dihydroxy-2-cyclohexen-1-one (**2**), and 4(*R*),5(*R*)-dihydroxy-2-cyclohexen-1-one **3** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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