Synthesis of Gallic Acid: Cu^{2+}-Mediated Oxidation of 3-Dehydroshikimic Acid

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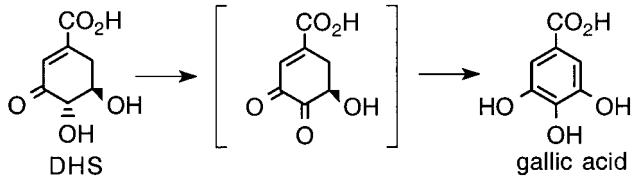
With the elaboration of high-yielding, high-titer syntheses of 3-dehydroshikimic acid from glucose using recombinant *Escherichia coli*, oxidation of this hydroaromatic becomes a potential route for synthesis of gallic acid. Conversion of 3-dehydroshikimic acid into gallic acid likely proceeds via initial enolization of an α-hydroxycarbonyl and oxidation of the resulting enediol. 3-Dehydroshikimate enolization in water was catalyzed by inorganic phosphate while Zn^{2+} was used to catalyze enolization in acetic acid. Enediol oxidation employed Cu^{2+} as either the stoichiometric oxidant or as a catalyst in the presence of a cooxidant. Gallic acid was produced in a yield of 36% when 3-dehydroshikimic acid in phosphate-buffered water reacted for 35 h with H_2O_2 and catalytic amounts of $CuSO_4$. 3-Dehydroshikimate-containing, phosphate-buffered culture supernatants reacted with stoichiometric amounts of $CuCO_3Cu(OH)_2$ and $Cu_x(H_{3-x}PO_4)_2$ to give gallic acid in yields of 51% in 5 h and 43% in 12 h, respectively. Solutions of 3-dehydroshikimic acid in acetic acid reacted with stoichiometric amounts of $Cu(OAc)_2$ to afford a 74% yield of gallic acid in 36 h. Acetic acid solutions of 3-dehydroshikimic acid could also be oxidized by air using catalytic quantities of $Cu(OAc)_2$. ZnO accelerated these oxidations leading to a 67% yield of gallic acid in 4 h when an acetic acid solution of 3-dehydroshikimic acid was reacted with O_2 and a catalytic amount of $Cu(OAc)_2$.

3-Dehydroshikimic acid (DHS, Scheme 1) is a hydroaromatic intermediate in the common pathway of aromatic amino acid biosynthesis and a key intermediate in the biocatalytic synthesis of vanillin,[1] catechol,[2] and adipic acid.[3] Although DHS is typically present in nature only in trace quantities, recombinant *Escherichia coli* have recently been constructed that are capable of synthesizing DHS from glucose in 30% yield and at concentrations of 69 g/L.[4] This drastic increase in the availability of DHS provides an opportunity to expand the range of molecules synthesized via DHS intermediacy by integrating chemical catalysis with microbe-catalyzed synthesis of DHS. A prime candidate for such a strategy is gallic acid, which the pioneering studies of Haslam demonstrated could be formed when DHS was reacted with Fehling solution.[5] Gallic acid is an important starting material used in the synthesis of a wide range of food additives and pharmaceuticals and is currently obtained from hydrolysis of gallotannins isolated from gall nuts and tara powder.[6]

DHS possesses α-hydroxycarbonyl functionality (Scheme 1), which is precedented to undergo selective, Cu^{2+}-mediated oxidation.[7,8] The α-dicarbonyl species (Scheme 1) resulting from Cu^{2+}-mediated oxidation of DHS would be expected to rapidly enolize to form gallic acid. Initial strategies focused on the use of inorganic phosphate to catalyze Cu^{2+}-mediated oxidation of DHS in aqueous solutions. Attention then turned to oxidations of DHS in AcOH solutions using either stoichiometric amounts of Cu^{2+} as the oxidant or catalytic amounts of Cu^{2+} with O_2 serving as the oxidant. Nonoxidative Lewis acids such as Zn^{2+} were discovered to accelerate the rate that acetic acid solutions of DHS were oxidized by O_2 in the presence of catalytic quantities of Cu^{2+}. Beyond the utility of increasing gallic acid availability by elaboration of a hybrid biocatalytic/chemical synthesis from glucose, oxidation of microbe-synthesized DHS provides a useful opportunity for comparison of oxidations employing stoichiometric versus catalytic quantities of a metal.

Scheme 1



Results

Isolation of Microbe-Synthesized DHS. Two different methods were effective for isolation of DHS from

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(1) Li, K.; Frost, J. W. *J. Am. Chem. Soc.* **1998**, *120*, 10545.
(2) Draths, K. M.; Frost, J. W. *J. Am. Chem. Soc.* **1995**, *117*, 2395.
(3) Draths, K. M.; Frost, J. W. *J. Am. Chem. Soc.* **1994**, *116*, 399.
(4) Li, K.; Mikola, M. R.; Draths, K. M.; Worden, R. M.; Frost, J. W. *Biotechnol. Bioeng.* **1999**, *64*, 61.
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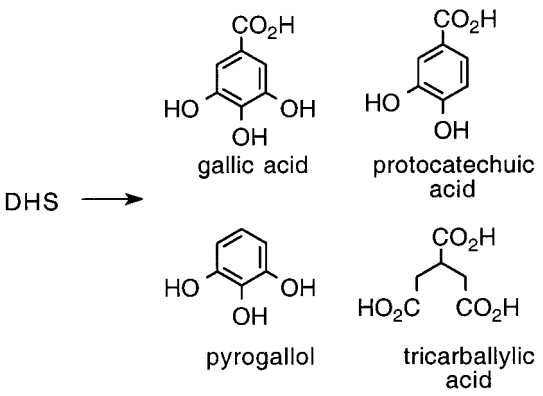
Table 1. Aqueous, Phosphate-Catalyzed Reactions of DHS[a,b]

		yield (%)					
oxidant	Cu (equiv)	GA	PCA	DHS	PGL	TCBA	time (h)
air	0	14	12	10	3	14	50
H_2O_2	0	22	10	20		2	36
H_2O_2	0.01	36	3				35

[a] Abbreviations: GA, gallic acid; PCA, protocatechuic acid; DHS, 3-dehydroshikimic acid; PGL, pyrogallol; TCBA, tricarballylic acid. [b] All reactions were run at 40 °C.

Scheme 2



microbial culture supernatants. The first method relied on liquid−liquid extraction while the second method was based on ion-exchange interactions. For both isolation methods, cells were removed by centrifugation, and the resulting cell-free supernatant was acidified. Precipitated protein was removed by a second centrifugation to afford the culture supernatant from which DHS was isolated. Continuous, liquid−liquid extraction using EtOAc resulted in a 95% recovery of the DHS from the culture supernatant. An 85% recovery of DHS was realized by loading culture supernatant on AG1 × 8 (AcO^-) anion-exchange resin followed by elution with glacial AcOH. Dehydration of the DHS to protocatechuic acid was not observed, and since AcOH was the solvent employed for many of the Cu^{2+}-mediated oxidations, eluted DHS could be directly oxidized.

Aqueous, Cu^{+2}-Mediated DHS Oxidations Catalyzed by Inorganic Phosphate. Reaction of DHS with air in phosphate-buffered water leads (Table 1) to the formation (Scheme 2) of gallic acid, protocatechuic acid, pyrogallol, and tricarballylic acid.[9] Reaction of DHS purified from culture supernatant with H_2O_2 in phosphate-buffered water gave (Table 1) higher yields of gallic acid relative to protocatechuic acid and tricarballylic acid with no observable formation of pyrogallol. Subsequently, it was discovered (Table 1) that addition of catalytic amounts (1% mol/mol relative to DHS) of $CuSO_4$ to the reactions of DHS with H_2O_2 in phosphate-buffered water eliminated tricarballylic acid formation, increased the yield of gallic acid, and decreased protocatechuic acid formation.

Heterogeneous oxidation of DHS in phosphate-buffered (1.0 M, pH 6.5) culture supernatant using stoichiometric amounts of $CuCO_3{\cdot}Cu(OH)_2$ led to faster reaction rates and higher yields of gallic acid (Table 2, entry 1). IR analysis of the copper salts recovered after DHS oxidation using $CuCO_3{\cdot}Cu(OH)_2$ showed absorbances indicative of phosphate rather than carbonate as the counteranion. Given anticipated problems with exchange of a carbonate for a phosphate counteranion during recycling of the Cu^{2+} oxidant, heterogeneous oxidation of DHS in phosphate-buffered (1 M, pH 6.6) culture supernatant using stoichiometric concentrations of $Cu_x(H_{3-x}PO_4)_2$ was explored. Similar yields of gallic acid and protocatechuic acid were observed when DHS was oxidized using $Cu_x(H_{3-x}PO_4)_2$ (Table 2, entry 3) relative to DHS oxidation using $CuCO_3{\cdot}Cu(OH)_2$ (Table 2, entry 1). Reducing the high concentrations of inorganic phosphate during heterogeneous oxidation of DHS using stoichiometric amounts of $CuCO_3{\cdot}Cu(OH)_2$ or $Cu_x(H_{3-x}PO_4)_2$ led to significantly slower reaction rates and significant concentrations of unreacted DHS (Table 2, entry 2 versus entry 1; entry 4 versus entry 3).

Table 2. Aqueous, Phosphate-Catalyzed Oxidation of DHS Using Stoichiometric Amounts of Cu^{2+} [a,b]

			yield (%)				
entry	oxidant	Pi (M)	Cu (equiv)	GA	PCA	DHS	time (h)
1	$CuCO_3Cu(OH)_2$	1.0	4	51	2		5
2	$CuCO_3Cu(OH)_2$	0.25	4	47	4	21	20
3	$Cu_x(H_{3-x}PO_4)_2$	1.0	2.2	43	2		12
4	$Cu_x(H_{3-x}PO_4)_2$	0.25	2.2	31	3	34	23

[a] Abbreviations: GA, gallic acid; PCA, protocatechuic acid; DHS, 3-dehydroshikimic acid. [b] All reactions were run at 50 °C.

Table 3. Oxidation of DHS in AcOH Using Catalytic and Stoichiometric Amounts of Cu^{2+} [a]

		yield (%)					
entry	catalyst	Cu (equiv)	GA	PCA	DHS	time (h)	*T* (°C)
1	$Cu(OAc)_2$	2.2	53	3		12	50
2	$Cu(OAc)_2$	2.2	74	0.7		36	40
3	$Cu(OAc)_2/NH_4NO_3$	0.05	38	2	5	24	40
4	$Cu(OAc)_2/H_2O_2$	0.1	21	4.3	34	18	40
5	$Cu(OAc)_2$/Air	0.1	48	2.5	30	23	40

[a] Abbreviations: GA, gallic acid; PCA, protocatechuic acid; DHS, 3-dehydroshikimic acid.

Cu^{2+}-Mediated DHS Oxidations In AcOH. High concentrations of inorganic phosphate were avoided when DHS purified from culture supernatant was dissolved in $AcOH/H_2O$ (3/1, v/v) and oxidized at 50 °C under homogeneous reaction conditions using stoichiometric (2.2 equiv) amounts of $Cu(OAc)_2$ (Table 3, entry 1). Water had to be included with the AcOH reaction solvent in order to dissolve the amounts of $Cu(OAc)_2$ required for stoichiometric oxidations. These reaction conditions were apparently quite sensitive to temperature. Although the rate of reaction was significantly slower, higher yields of gallic acid and only trace concentrations of protocatechuic acid were observable when DHS dissolved in $AcOH/H_2O$ (3/1, v/v) was oxidized by $Cu(OAc)_2$ at 40 °C (Table 3, entry 2).

To determine whether catalytic amounts (0.1 equiv) of $Cu(OAc)_2$ could be employed during oxidation of DHS to gallic acid, use of NH_4NO_3, H_2O_2 and air as oxidant were examined (Table 3, entries 3−5). Higher yields of gallic acid were obtained when air was the oxidant relative to use of H_2O_2 or NH_4NO_3 as the oxidant. This difference in gallic acid yield prompted an appraisal of gallic acid stability under oxidative conditions. Since the lowest yields of gallic acid were obtained during Cu^{2+}-catalyzed oxidation using H_2O_2, this reaction was examined for

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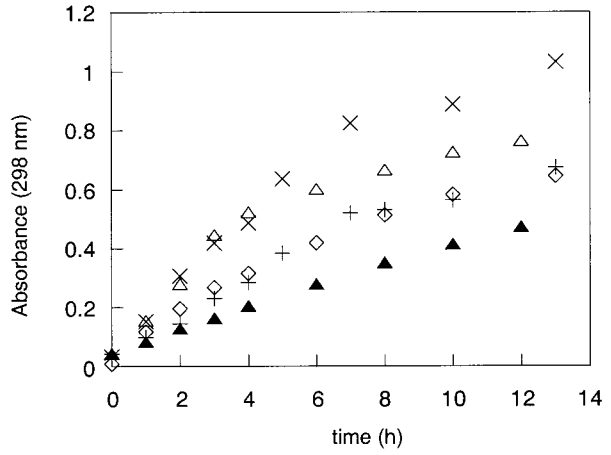
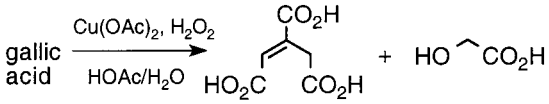


Figure 1. Rate of reaction at 50 °C of DHS (24 mM) in AcOH with 10 mol % Cu^{2+} and: ×, O_2/50 mol% Zn^{+2}; △, air/50 mol% Zn^{+2}; +, air/50 mol% Mn^{+2}; ◇, air/50 mol% Mg^{+2}; ▲, no added Lewis acid.

Scheme 3



products resulting from overoxidation of gallic acid. With catalytic amounts of $Cu(OAc)_2$ and reaction conditions similar to those employed for DHS oxidation, H_2O_2 oxidation resulted in complete consumption of gallic acid in 5 h and formation of a 20% yield of aconitic acid and a 10% yield of hydroxyacetic acid (Scheme 3). Neither aconitic acid or hydroxyacetic acid were formed when oxidation of gallic acid by air using catalytic amounts of $Cu(OAc)_2$ was attempted. Approximately 95% of the starting gallic acid remained unreacted after 16 h.

Cu^{+2}-Mediated DHS Oxidations in AcOH Catalyzed by Lewis Acids. In an attempt to accelerate the rate of DHS oxidation using catalytic amounts of $Cu(OAc)_2$, the impact of adding nonoxidative Lewis acids to the reaction was examined. All oxidations were run at 50 °C under 1 atm of either air or O_2 with DHS concentrations at 0.05 M. Relative to the DHS, 0.1 equiv of $Cu(OAc)_2$ and 0.5 equivalents of ZnO, MgO, and MnO were employed. At 19/1 (mol/mol), the ratio of gallic acid/protocatechuic acid formed during DHS oxidation was independent of the added Lewis acid. The rate of DHS oxidation was significantly increased (Figure 1) by all of the nonoxidative Lewis acids examined with the greatest rate acceleration associated with the presence of Zn^{2+}. An additional acceleration in the overall rate of DHS oxidation (Figure 1) was observed when Zn^{2+} oxidations were run under O_2 instead of air.

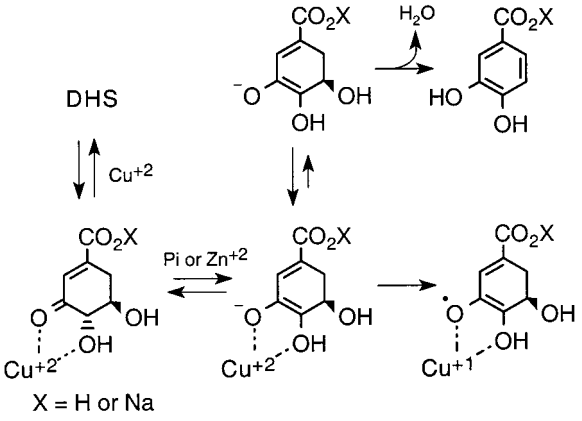
DHS was quite stable in AcOH solutions heated to 50 °C in the absence of added Cu^{2+} or Zn^{2+}. As opposed to inorganic phosphate-buffered solutions of DHS (Table 1, entry 1), 90% of the DHS in AcOH/H_2O (85:15, vol/vol) solutions exposed to air remained unreacted (Table 4, entry 1) with formation of only trace amounts of gallic and protocatechuic acids. Addition of Zn^{2+} (0.5 equivalents relative to DHS) in lieu of added Cu^{2+} led to an 80% yield (Table 4, entry 2) of protocatechuic acid. A 73% yield of gallic acid was realized (Table 4, entry 3) with Cu^{2+}-mediated reaction of DHS (0.05 M) with air in the presence of Zn^{2+} (0.5 equiv relative to DHS). The number

Table 4. Zn^{2+}-Catalyzed Oxidation of DHS in AcOH Using Catalytic Amounts of Cu^{+2} [a,b]

					yield (%)			
entry	DHS (M)	cooxidant	Cu (equiv)	Zn (equiv)	GA	PCA	DHS	time (h)
1	0.05	air			trace	trace	90	14
2	0.05	air		0.5	1	80	5	16
3	0.05	air	0.1	0.5	73	4		23
4	0.05	air	0.1	0.1	71	5	20	23
5	0.5	air	0.05	0.1	70	3	11	7
6	1	air	0.05	0.1	46	14	8	7
7	1	O_2	0.05	0.1	64	3.5	9	6
8	1	O_2	0.05	0.2	67	3		4

[a] Abbreviations: GA, gallic acid; PCA, protocatechuic acid; DHS, 3-dehydroshikimic acid. [b] All reactions were run at 50 °C.

Scheme 4



of Zn^{2+} equivalents could be reduced by 5-fold without reducing the yield of gallic acid or increasing reaction times (Table 4, entry 4 versus entry 3).

Further increases in the rate of Cu^{2+}-mediated oxidation by air in the presence of Zn^{2+} were achieved by increasing the concentration of DHS by 10-fold (Table 4, entry 5). However, additional increases in the concentration of DHS resulted in a marked decline in the yield of gallic acid and an increase in the yield of protocatechuic acid (Table 4, entry 6). This reduction in the yield of product gallic acid was reversed upon use of O_2 instead of air (Table 4, entry 7). At 1 M concentrations of DHS with use of O_2, increasing the Zn^{2+} concentration increased the rate of the reaction but did not significantly increase the yield of gallic acid. (Table 4, entry 8 versus entry 7).

Discussion

Mechanistic Considerations. Oxidation of DHS to form gallic acid is an example of a larger class of oxidations involving conversion of α-hydroxycarbonyls to α-dicarbonyls using stoichiometric[7] or catalytic[8] amounts of Cu^{2+}. These oxidations likely begin with complexation of the α-hydroxycarbonyl to form a Cu^{2+}−ketol complex (Scheme 4).[10,11] Subsequent enolization of the α-hydroxycarbonyl species to an enediol (Scheme 4) is the rate-determining step for the overall oxidation.[11,12] This is based on the report of a large kinetic isotope effect[11] associated with Cu^{2+}-mediated oxidation of a dueterium-

(10) Pedersen, K. J. *Acta Chem. Scand.* **1948**, *2*, 253.
(11) Wiberg, K. B.; Nigh, W. G. *J. Am. Chem. Soc.* **1965**, *87*, 3849.
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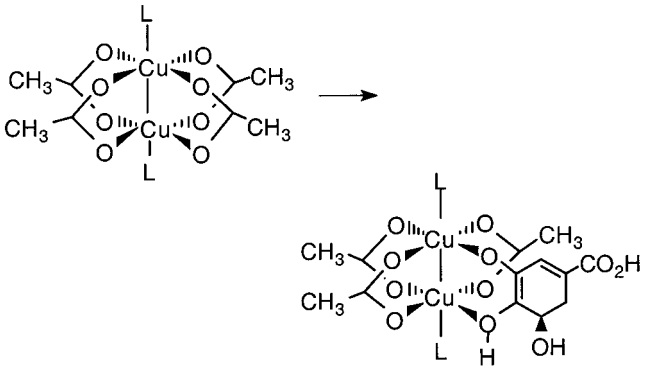
labeled ketol, and the first-order dependence[12] on base concentration during base-catalyzed oxidation of α-hydroxycarbonyls using Cu^{2+}. α-Hydroxycarbonyl enolization thus provides a convenient basis for mechanistic appraisal of the methods employed to oxidize DHS.

In aqueous solutions, the rate of oxidation of DHS using either stoichiometric amounts of Cu^{2+} or catalytic amounts of Cu^{2+} with O_2 functioning as the stoichiometric oxidant depends on the concentration of inorganic phosphate. This suggests that inorganic phosphate is catalyzing rate-determining enolization of DHS. Formation of gallic acid when DHS is dissolved in oxygenated, phosphate-buffered water has previously been demonstrated to be a general base-catalyzed process with inorganic phosphate functioning as the base.[9] Phosphate-catalyzed oxidation of DHS also occurs when H_2O_2 is employed as the oxidant. Upon addition of even catalytic quantities of Cu^{2+} to the oxidation of DHS using H_2O_2, gallic acid becomes the predominant product. This switch in the ratio of products is consistent with the Cu^{2+} complexing with the ketol prior to enolization (Scheme 4). Resulting stabilization of the enediol as it is formed would likely direct the reaction coordinate away from dehydration (Scheme 4) and attendant formation of protocatechuic acid.

In acetic acid solutions containing water, DHS oxidations employ either a stoichiometric quantity of Cu^{2+} or a catalytic quantity of Cu^{2+} in combination with a nonoxidative Lewis acid catalyst. Use of a stoichiometric amount of Cu^{2+} enables the oxidation of DHS to take advantage of this cation's ability to catalyze enolization of the α-hydroxycarbonyl.[10] The pronounced catalytic roles played by nonoxidative Lewis acids such as Zn^{2+} during oxidation of DHS using catalytic amounts of Cu^{2+} has not previously been reported. Enolization catalyzed by Zn^{2+} may reflect formation of a ternary Cu^{2+}-enediol-Zn^{2+} complex where Zn^{2+} binds to the diene portion of an initially formed Cu^{2+}-enediol complex. Zn^{2+}-diene complexes involving both $\sigma-$ and π-type interactions are known to form.[13] It is also possible that Zn^{2+} may initially complex with the α-hydroxycarbonyl and catalyze enolization resulting in formation of a Zn^{2+}-enediol complex. To avoid formation of uncomplexed enediol and dehydration resulting in formation of protocatechuic acid (Scheme 4), Cu^{+2} would likely need to bind prior to dissociation of Zn^{2+}.

$Cu(OAc)_2$ forms both monomeric and dimeric complexes with carboxylic acids with the dimeric complex (Scheme 5) known to dominate in acetic acid solutions.[14] This dimeric complex may play an important role in Cu^{2+}-mediated oxidations in AcOH solutions. Displacement of one of the acetic acid ligands by DHS (Scheme 5) would allow the two Cu^{2+} ions required for oxidation of the enediol to the α-dicarbonyl to be in close proximity to one another and the functionality undergoing oxidation. Increasing the concentration of water would be expected to disrupt formation of the dimeric copper complex.[8a] This may account for the observed decrease in DHS oxidation rates as the percentage of water was increased beyond

Scheme 5



an 85:15 (v/v) ratio of AcOH/H_2O. Inhibition of oxidation has also been observed upon addition of water to steroidal α-hydroxycarbonyls.[8a] The formation of Cu^{2+} dimers in phosphate-buffered, aqueous solutions is uncertain given the expected disruption of Cu^{2+} dimerization by water and inorganic phosphate.

Synthetic Considerations. Our experience using Cu^{2+}-containing Fehling solution as reported by Haslam[5] to oxidize DHS indicated that gallic acid was produced in low yield concurrent with formation of high levels of protocatechuic acid contamination. Nonetheless, Haslam's observations[5] prompted us to add catalytic quantities of $CuSO_4$ to phosphate-buffered solutions of DHS. The resulting improvements in gallic acid synthesis (Table 1) when H_2O_2 was used as the oxidant led to our evaluation of using Cu^{2+} as both a catalytic and stoichiometric oxidant. Observations that inorganic phosphate in aqueous solutions and ZnO in AcOH solutions could be used to catalyze the oxidation of DHS to gallic acid introduces two new variables that can be manipulated during the use of Cu^{2+} for oxidation of α-hydroxycarbonyls.

Cu^{2+}-based oxidation of DHS in aqueous solution using inorganic phosphate catalysis minimizes the number of manipulations that are required between microbe-catalyzed synthesis of DHS from glucose and oxidation of this hydroaromatic. DHS does not need to be isolated from the culture supernatant. Heterogeneous oxidation conditions using $CuCO_3Cu(OH)_2$ or $Cu_x(H_{3-x}PO_4)_2$ gave comparable yields of gallic acid and similar levels of protocatechuic acid contamination. Catalytic amounts of Cu^{2+} could also be employed for oxidation of DHS in phosphate-buffered, aqueous solution. However, O_2 could not be used as the stoichiometric oxidant. The need to use H_2O_2 and the resulting modest yields of gallic acid can likely be attributed to overoxidation of gallic acid by H_2O_2. Oxidation of DHS in aqueous solution and culture supernatant using either stoichiometric or catalytic amounts of Cu^{2+} required high concentrations (1 M) of inorganic phosphate. Such high-salt reaction conditions would be problematic from a disposal standpoint if they were employed on a large scale.

Cu^{2+}-based oxidation of DHS to gallic acid in AcOH solution required the use of either continuous liquid−liquid extraction or an anion-exchange resin for isolation of DHS from culture supernatants. Although this increases the number of manipulations required for synthesis of gallic acid from microbe-synthesized DHS, high-salt reaction conditions are avoided and the yields of gallic acid using Cu^{2+}-based oxidations in AcOH are

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(14) (a) van Niekerk, J. N.; Schoening, F. R. L. *Acta Crystallogr.* **1953**, *6*, 227. (b) Kato, M.; Jonassen, H. B.; Fanning, J. C. *Chem. Rev.* **1964**, *64*, 99.

higher than Cu^{2+}-based oxidations run in aqueous environments. An additional advantage of using AcOH for DHS oxidation is that catalytic quantities of $Cu(OAc)_2$ can be used with O_2 serving as the cooxidant. Irrespective of whether stoichiometric amounts of $Cu(OAc)_2$ or catalytic amounts of $Cu(OAc)_2$ were used in the presence of ZnO, the yields of gallic acid resulting from oxidation of DHS in AcOH solution were approximately the same. Oxidation of DHS using stoichiometric amounts of $Cu(OAc)_2$ did give significantly lower levels of protocatechuic acid byproduct formation relative to DHS oxidation using O_2 catalyzed by $Cu(OAc)_2$ and ZnO.

Oxidation of DHS catalyzed by $Cu(OAc)_2$ and ZnO is consonant with the trend toward development of Cu-catalyzed oxidations of alcohols using O_2 as the oxidant.[15] Oxidation of DHS by O_2 using catalytic (5 mol %) amounts of $Cu(OAc)_2$ required significant amounts (10−20 mol %) of ZnO in order to achieve sizable rate accelerations. Nonetheless, this constitutes a sizable reduction in total metal requirement relative to oxidation of DHS using stoichiometric amounts (220 mol %) of $Cu(OAc)_2$. Recovery and recycling of metals also needs to be considered. After DHS oxidation using stoichiometric amounts of $Cu(OAc)_2$, the Cu^{1+} was oxidized by H_2O_2 and the resulting $Cu(OH)_2$ precipitated upon basification. Dissolving the filtered precipitate in AcOH, concentration, and drying resulted in the recovery of 93% of the initially used $Cu(OAc)_2$. A straightforward method for high-yielding recovery and recycling of both Cu^{1+} and Zn^{2+} was not identified for oxidation of DHS catalyzed by $Cu(OAc)_2$ and ZnO. As a result, the 10-fold lowering in metal equivalents required to oxidize DHS using O_2 as the oxidant relative to DHS oxidation using stoichiometric amounts of $Cu(OAc)_2$ will not be fully realized until a successful procedure for total metal recovery and recycling is elaborated.

Experimental Section

General Chemistry. ^{1}H NMR spectra were recorded on a 300 MHz spectrometer. Chemical shifts were reported in parts per million (ppm) downfield from internal sodium 3-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$ (TSP, $\delta = 0.00$) when D_2O was the solvent. TSP was purchased from Lancaster. Octadecyl-functionalized silica gel was purchased from Aldrich and activated prior to use by elution with MeOH. AG-1 × 8 (AcO^-) anion-exchange resin and Dowex 50 (H^+) cation-exchange resin were purchased from Bio-Rad.

Liquid−Liquid Extraction of DHS from Culture Supernatant. KL3/pKL4.79B was cultured under fed-batch fermentor conditions according to ref 4. Fermentation broth was centrifuged (3000*g* for 10 min) to remove cells and the resulting culture supernatant (500 mL) containing DHS (15.5 g, 0.18 M) acidified to pH 2 with addition of concentrated HCl. Precipitated protein was removed by centrifugation (13000*g* for 10 min). The resulting black solution was then stirred in a continuous liquid−liquid extraction apparatus at a rate to create a translucent colloidal suspension of EtOAc in the aqueous culture supernatant while allowing the colloidal suspension to separate into a clear organic phase at the top of the extraction cylinder. DHS-containing EtOAc was replaced with fresh EtOAc (400 mL) at 2, 5, 8 and 12 h. After the 2 L of DHS-contaning EtOAc was filtered through 10−15 g of Darco G-60 (100 mesh) activated charcoal and dried over $MgSO_4$, the yellow-colored solution was concentrated to a volume of 50−60 mL. Chilling this concentrated solution in an ice bath led to the precipitation of DHS as an off-white powder (10.5 g, 68%).

Resin-Based Isolation of DHS from Culture Supernatant. KL3/pKL4.66A was cultured under fed-batch fermentor conditions according to ref 4. Fermentation broth was centrifuged (3000*g* for 10 min) to remove cells and the resulting culture supernatant (900 mL) containing DHS (17 g, 0.11 M) acidified to pH 2with addition of concentrated HCl. Precipitated protein was removed by centrifugation (13000*g* for 10 min). The resulting black-colored solution was adjusted to pH 5 with addition of NaOH and then applied to a column containing 400 mL of AG-1 × 8 (AcO^-). After washing the column with H_2O (600 mL) and MeOH (600 mL), decolorized DHS (14.5 g, 85%) was selectively eluted with glacial AcOH (500 mL). The acetate form of the resin was regenerated by eluting it with NaOH (0.1 N) followed by elution with HOAc/H_2O (v/v, 1/1).

$CuSO_4$-Catalyzed Oxidation of DHS by H_2O_2 in Phosphate-Buffered H_2O. DHS (1 g, 5.88 mmol), $CuSO_4 \cdot H_2O$ (0.0147 g, 0.0588 mmol), $NaH_2PO_4 \cdot H_2O$ (4.14 g, 30 mmol), and Na_2HPO_4 (4.26 g, 30 mmol) were dissolved in 49 mL of H_2O. After adjusting the solution to pH 6.6, 11 mL of a 2% solution of H_2O_2 (6.40 mmol) was added and the reaction stirred at 40 °C for 35 h. The reaction solution was then adjusted to pH 2.5 by addition of H_2SO_4, extracted with EtOAc (4 × 50 mL), and concentrated to a solid consisting of gallic acid (36%) and protocatechuic acid (3%). Chromatography on octadecyl-functionalized silica gel (H_2O/MeOH, 9:1, v/v adjusted to pH 2.5 with AcOH) provided gallic acid (0.31 g, 31%), which was free of protocatechuic acid contamination.

Oxidation of DHS by $CuCO_3 \cdot Cu(OH)_2$ in Phosphate-Buffered H_2O. *E. coli* AB2834/pMF63A was cultured in shake flasks as described in ref 2, and cells were removed from the culture broth by centrifugation (3000*g* for 10 min) to provide a culture supernatant (1 L) containing DHS (7.9 g, 46 mmol). $KH_2PO_4 \cdot H_2O$ (68 g, 500 mmol) and K_2HPO_4 (87 g, 500 mmol) were then added and the culture supernatant adjusted to pH 6.5. Addition of $CuCO_3 \cdot Cu(OH)_2$ (20.3 g, 92 mmol) resulted in a heterogeneous solution, which was vigorously stirred at 50 °C for 5 h. After filtration to remove insoluble copper salts, the solution was acidified to pH 2.5 with addition of concentrated H_2SO_4 and extracted with EtOAc. Concentration of the organic layer gave a red solid, which was dissolved in 150 mL of H_2O/MeOH (9:1, v/v) and filtered through octadecyl-functionalized silica gel. Concentration of the filtrate to 30 mL resulted in formation of a precipitate. Filtration and drying afforded 4.1 g of an off-white solid consisting of gallic acid (51%) and protocatechuic acid (2%).

Oxidation of DHS by $Cu_x(H_{3-x}PO_4)_2$ in Phosphate-Buffered H_2O. *E. coli* AB2834/pMF63A was cultured in shake flasks as described in ref 2 and cells removed from the culture broth by centrifugation. KH_2PO_4 (13.6 g) was added to a portion (100 mL) of this culture supernatant containing DHS (0.79 g, 4.6 mmol) and the solution adjusted to pH 6.6 by addition of NaOH. The $Cu_x(H_{3-x}PO_4)_2$ oxidant was prepared by dissolving $CuSO_4$ (1.6 g, 10 mmol in 50 mL of H_2O followed by addition of $NaHPO_4$ (2.2 g, 0.016 mol). The blue-colored precipitate that formed was recovered by filtration and then added to the reaction solution. This heterogeneous solution was stirred under N_2 at 50 °C for 12 h. After filtration to remove the insoluble copper salts, the reaction solution was acidified to pH 2.2 with addition of H_2SO_4 and extracted with EtOAc (3 × 60 mL). Concentration of the organic layer afforded 0.35 g of a lightly yellow-colored solid consisting of gallic acid (43%) and protocatechuic acid (2%).

Oxidation of DHS by $Cu(OAc)_2$ in AcOH. A solution of DHS (5.4 g, 31.4 mmol) and $Cu(OAc)_2$ (13.5 g, 67.5 mmol) in 400 mL of AcOH/H_2O (3:1, v/v) was stirred at 40 °C under N_2 for 36 h. After filtration to remove the insoluble copper salts, the reaction solution was concentrated almost to dryness and then dissolved in H_2O (300 mL). This aqueous solution was extracted with EtOAc (4 × 100 mL) and the organic layer concentrated. The resulting brown solid consisting of gallic acid (74%) and protocatechuic acid (0.7%) was dissolved in H_2O/MeOH (9:1, v/v) and decolorized by filtration through octade-

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cyl-functionalized silica gel. During concentration of the filtrate, gallic acid precipitated and was recovered as an off-white solid (3.3 g, 62%). Recovery of copper salts and recycling of these salts back to $Cu(OAc)_2$ began with combining the filtered copper salts with the aqueous layer after removal of gallic acid by EtOAc extraction. This mixture was reacted overnight with 10 mL of 30% H_2O_2 at room temperature. Addition of solid NaOH to give a solution of pH 13 resulted in precipitation of $Cu(OH)_2$, which was recovered by filtration. Stirring the $Cu(OH)_2$ in AcOH for 2 h led to a homogeneous solution that after concentration and drying gave 12.5 g of $Cu(OAc)_2$ (93%) indistinguishable by IR from the $Cu(OAc)_2$ initially used to oxidize DHS in the culture supernatant.

ZnO, $Cu(OAc)_2$-Catalyzed Oxidation of DHS by O_2 in AcOH. Oxygen was bubbled through a stirred solution of DHS (10.5 g, 61.0 mmol), $Cu(OAc)_2$ (0.61 g, 3.05 mmol), and ZnO (0.995 g, 12.2 mmol) in 61 mL of AcOH/H_2O (85:15, v/v) at 50 °C for 4 h. Gallic acid (67%) and protocatechuic acid (3%) were the only products formed. The reaction solution was concentrated to 30 mL, chilled in an ice bath, and the black solid that formed collected by filtration. This solid was dissolved in H_2O (250 mL) and the resulting solution extracted with EtOAc (4 × 150 mL). The combined organic layers were concentrated to 200 mL and chilled in an ice bath. Petroleum ether (200 mL) was then added. A precipitate formed which was filtered and dried affording 5.5 g (53%) of gallic acid as a slightly yellow, off-white solid. Further decolorization was accomplished by dissolving the gallic acid in 300 mL H_2O/MeOH (9:1, v/v) followed by filtration through octadecyl-functionalized silica gel. Upon concentration of the decolorized solution to 30 mL a solid formed which was filtered and dried to give 5.1 (49%) of gallic acid as a white solid.

Zn^{2+}, Mg^{2+}, Mn^{2+} Acceleration of Cu^{2+}-Catalyzed DHS Oxidations. DHS (0.1 g, 0.58 mmol) and $Cu(OAc)_2$ (0.012 g, 0.058 mmol) were dissolved in 24 mL of AcOH/H_2O (85:15, v/v) containing ZnO (0.23 g, 0.29 mmol), MgO (0.012 g, 0.23 mmol), or MnO (0.29 g, 0.29 mmol). Reaction progress at 50 °C under either air or O_2 was determined by withdrawing 0.1 mL aliquots from the reaction solution at timed intervals, diluting with AcOH, and measuring the absorbance at 298 nm for each time interval. To verify that the absorbance at 298 nm corresponded to the concentration of gallic and protocatechuic acids, 1 mL aliquots of the reaction solution at various timed intervals were filtered through a pipet containing 0.3 mL of Dowex 50 (H^+) to remove metals. These solutions were concentrated, dissolved in D_2O, a known amount of TSP added, and the concentration of gallic acid and protocatechuic acids determined by ^1H NMR.

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