Table I. One-Pot Synthesis of (*S*)-Cyanohydrin Acetates **3a–g** from Aldehydes **1a–g** Catalyzed by Lipase and Anion-Exchange Resin (OH^- Form)[a]

aldehydes	anion-exchange resin	reaction time (days)	conversion[b]		(*S*)-acetate **3**	
			1 to **2** (%)	**2** to **3** (%)	yield[c] (%)	ee[d] (%)
1a	Amberlite IRA-904	2.9	95	88	80	89
1a	Amberlyst A-27	2.9	90	77	68	88
1a	Duolite A-162	2.1	97	93	82	80
1b	Amberlite IRA-904	6.3	100	100	96	84
1c	Amberlite IRA-904	3.8	97	93	83	84
1d	Amberlite IRA-904	2.5	88	82	64	91
1e	Amberlite IRA-904	6.5	89	96	81	91
1f	Amberlite IRA-904	6.1	98	98	88	85
1g	Amberlite IRA-904	6.1	91	79	70	70

[a] Typical conditions: 3-phenoxybenzaldehyde (**1a**) (991 mg, 5.0 mmol), acetone cyanohydrin (851 mg, 10.0 mmol), isopropenyl acetate (1502 mg, 15.0 mmol), lipase (500 mg), anion-exchange resin Amberlite IRA-904 (OH^- form, 192 mg, 10 mol % equiv to **1a**), 3 Å ground molecular sieves (200 mg), dry diisopropyl ether (40 mL), 40 °C. The addition of 3 Å molecular sieves accelerated the reaction although 3 Å MS catalyzes neither the transhydrocyanation nor the acetylation. [b] Determined by ^1H NMR. [c] Isolated yield from aldehyde **1**. [d] Determined by ^1H NMR in the presence of the chiral shift reagent, Eu(hfc)$_3$.

By comparing the sign of the optical rotation with that reported,[11] the absolute configuration of **3a** was found to be *S*. The ee was determined to be 89% by ^1H NMR in the presence of a chiral shift reagent.

An initial approach using quinidine or quinine as a catalyst for transhydrocyanation resulted in a long reaction time as well as a low optical yield of **3a**,[12] presumably due to slow racemization of the cyanohydrins. Consequently, a stronger base was required as a catalyst for the present process. The OH^- form of anion exchange resins, such as Amberlite IRA-904, is a strong base and was found to be an efficient catalyst for rapid transhydrocyanation between aromatic aldehyde and acetone cyanohydrin in an organic solvent.[13] Moreover, such resins are insoluble in the reaction solvent and are insulated completely from the lipase, causing no unfavorable effects on lipase activity. All of the three resin types listed in Table I effectively catalyzed the transhydrocyanation (racemization) to produce high chemical yields of (*S*)-**3a**. The somewhat low ee for **3a** in the reaction using Duolite A-162 may be attributable to the nonenzymatic acetylation of racemic **2a** catalyzed by this resin.[14]

Benzaldehyde **1b** was completely (100% yield) converted into acetate (*S*)-**3b** with an 84% ee. Similarly, optically active cyanohydrin acetates **3c–g** having a high optical purity (up to 91%) were obtained from the corresponding aldehydes **1c–g** in 64–88% isolated yields. All the acetates **3a–g** were of *S* configuration,[15] indicating that the lipase preferentially converted the *S* cyanohydrin into the corresponding acetate irrespective of the substituents on the aromatic ring. The *S* isomer of the cyanohydrin **2a** is the desired enantiomer for the preparation of pyrethroids which are characterized by high insecticidal activity.[16]

In order to effectively convert racemic **2** into optically active **3**, the rate of racemization must be faster than that of lipase-catalyzed acetylation. The half-life ($t_{1/2}$) of the racemization of (*R*)-**2a** was 74 min when 10 mol % of Amberlite IRA-904 (OH^- form) was used as a catalyst in the presence of acetone cyanohydrin in anhydrous diisopropyl ether at 25 °C,[17] which confirms that racemization is much faster than lipase-catalyzed acetylation.

Supplementary Material Available: Experimental details and analysis data (including ^1H NMR, IR, ^{13}C NMR, and MS), schemes showing the preparation of optically active **2c**, **2f**, and **2g**, and ^1H NMR spectra of **3a** (15 pages). Ordering information is given on any current masthead page.

(11) Smith, F. J.; Roper, J. M. *Jpn. Kokai Tokkyo Koho* JP 62,164,657 [87,164,657], 1987; *Chem. Abstr.* **1988**, *108*, P 110873t.

(12) When quinidine was used as a transhydrocyanation catalyst in the present reaction, (*S*)-**3a** with 62% ee was obtained in 71% conversion yield after 13.7 days. Cinchona alkaloids such as quinine and quinidine were used as catalysts for enantioselective addition of HCN to benzaldehyde. Prelog, V.; Wilhelm, M. *Helv. Chim. Acta* **1954**, *37*, 1634–1660.

(13) The strongly basic macroporous resin, Amberlite IRA-904 (OH^- form, 10 mol %), catalyzed the formation of cyanohydrin **2a** from 3-phenoxybenzaldehyde (**1a**) in the presence of 2 equiv mol of acetone cyanohydrin in anhydrous diisopropyl ether (82% conversion, 80 min, 40 °C). On the other hand, neither a weakly basic macroporous resin such as Amberlite IRA-35 (free base form) nor a strongly basic but gel-type resin such as Amberlite IRA-400 (OH^- form) catalyzed the reaction under the same reaction conditions.

(14) The nonenzymatic acetylation of (±)-**2a** catalyzed by anion-exchange resins was estimated in the absence of the lipase: Duolite A-162 (39%), Amberlite IRA-904 (8%), and Amberlyst A-27 (0%) after 2.9 days.

(15) The optical rotation values have been reported as follows: for **3d** and **3e**, ref 4d; for **2c**, **3f**, and **2g**, Mattews, B. R.; Jackson, W. R.; Jayatilake, G. S.; Wilshire, C.; Jacobs, H. A. *Aust. J. Chem.* **1988**, *41*, 1697–1709.

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(17) Optically active cyanohydrin **2a** (87% ee) was racemized completely in 6.2 h in the presence of 10 mol % of Amberlite IRA-904 and acetone cyanohydrin (10 equiv) in dry diisopropyl ether at 25 °C. Only 7% of **2a** was decomposed into the aldehyde **1a**. In the absence of acetone cyanohydrin, however, decomposition of cyanohydrin was observed (**2a**/**1a** = 75:25, equilibrium). The addition of acetone cyanohydrin prevented decomposition of **2a** and promoted clean racemization of **2a**.

Conversion of D-Glucose into Catechol: The Not-So-Common Pathway of Aromatic Biosynthesis

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Synthesis of catechol by *Escherichia coli* has led to the discovery of a pathway which can siphon away most of the D-glucose equivalents initially directed into the common pathway of aromatic biosynthesis (Scheme I). Induction of the discovered pathway constitutes an important variable to consider in the design of biocatalytic syntheses of aromatic amino acids and related secondary metabolites. The induced pathway may also be a useful route for converting D-glucose into catechol, a molecule from which a variety of pharmaceuticals, pesticides, flavors, and polymerization inhibitors are industrially derived.[1]

The percentage of D-glucose consumed by *E. coli* which is siphoned into aromatic biosynthesis is greatly increased when transketolase (*tkt*) and an isozyme of DAHP synthase (*aroF*) are amplified upon transformation with plasmid pKD136.[2] In addition to *tkt* and *aroF*, pKD136 carries an *aroB* locus which prevents accumulation of 3-deoxy-D-*arabino*-heptulosonic acid (DAH). Expression of pKD136 by *E. coli aroE* results in synthesis of a 30 mM concentration of 3-dehydroshikimate.[2b] By contrast,

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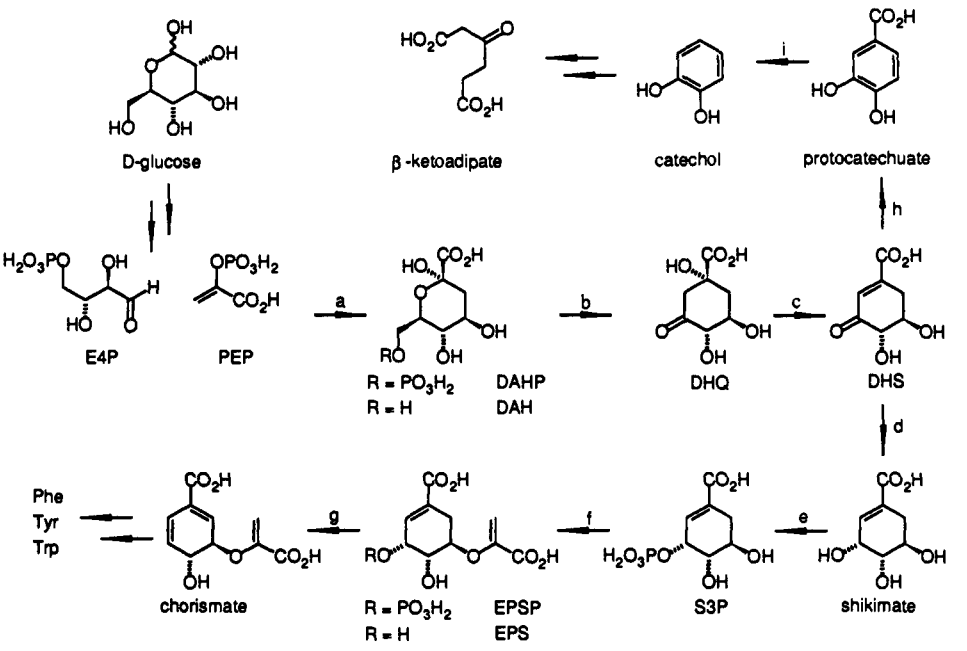
Scheme I[a]



[a] (a) DAHP synthase (*aroF*); (b) DHQ synthase (*aroB*); (c) DHQ dehydratase (*aroD*); (d) shikimate dehydrogenase (*aroE*); (e) shikimate kinase (*aroL*); (f) EPSP synthase (*aroA*); (g) chorismate synthase (*aroC*); (h) DHS dehydratase; (i) protocatechuate decarboxylase. Enzymes a–g are referred to as the common pathway of aromatic biosynthesis.

E. coli aroA/pKD136 accumulates only 2.4 mM shikimate 3-phosphate in its culture supernatant while *E. coli aroC*/pKD136 synthesizes a 3.8 mM concentration of 5-enolpyruvylshikimate 3-phosphate (EPSP) and 5-enolpyruvylshikimate (EPS). On the basis of the concentrations of metabolites accumulated by *aroE*, *aroA*, and *aroC* mutants of *E. coli*, approximately 90% of the D-glucose equivalents directed into aromatic biosynthesis are lost after DHS formation.

The first clue as to the fate of the lost D-glucose equivalents came with the detection of catechol[3] in concentrations up to 3.75 mM along with trace levels of protocatechuate in the culture supernatants of both *E. coli aroA*/pKD136 and *E. coli aroC*/pKD136. Formation of catechol and protocatechuate are consistent with induced expression of a pathway consisting of DHS dehydratase[4] and protocatechuate decarboxylase[5] (Scheme I). These enzymes have not previously been detected in *E. coli*. Furthermore, a drastic increase in the number of D-glucose equivalents directed into aromatic biosynthesis is not precedented to induce DHS dehydratase or protocatechuate decarboxylase in microbes known to possess these enzymes.[4,5]

Catechol concentrations accumulated by *E. coli aroA*/pKD136 and *E. coli aroC*/pKD136 could not entirely account for the loss of D-glucose equivalents. This inexplicable loss and the indication that DHS was the point of divergence for the putative pathway prompted a series of experiments with cell lysates prepared from a strain which is unable to convert DHS into shikimate (Scheme I). *E. coli aroE*/pKD136 cells were harvested and lysed when protocatechuate was detected in the culture supernatant. Analysis of cell lysate which had been incubated with DHS under nitrogen (Figure 1a) revealed that a large fraction of DHS had been converted into a mixture of catechol and protocatechuate. DHS dehydratase activity was also detected in cell lysates of *E. coli aroA*/pKD136 and *E. coli aroC*/pKD136.

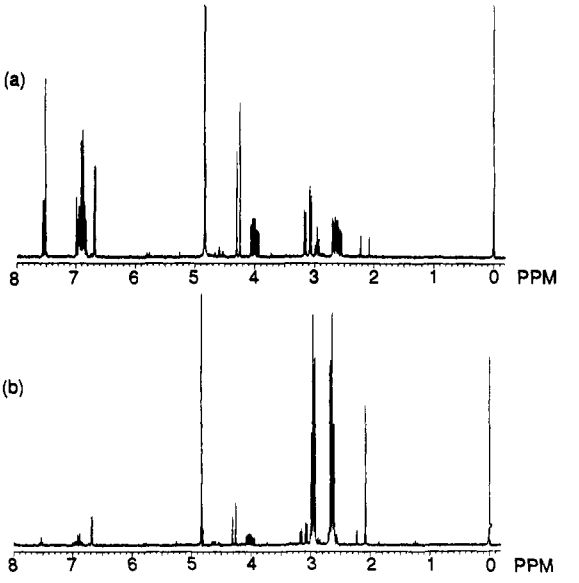


Figure 1. (a) ^{1}H NMR spectrum (D_2O) of the ethyl acetate extract of acidified (to pH 2.5) *E. coli aroE*/pKD136 cell lysate which had been incubated with DHS for 4 h at 37 °C under nitrogen. Resonances include those from unreacted DHS [δ 2.61 (ddd, J = 16, 10, 3 Hz, 1 H), 3.12 (dd, J = 18, 5 Hz, 1 H), 4.00 (ddd, J = 11, 10, 5 Hz, 1 H), 4.28 (d, J = 11 Hz, 1 H), 6.67 (d, J = 3 Hz, 1 H)] as well as product catechol [δ 6.82–6.95 (m, 4 H)] and product protocatechuic acid [δ 6.97 (dd, J = 7, 1 Hz, 1 H), 7.52 (d, J = 1 Hz, 1 H), 7.55 (dd, J = 7, 1 Hz, 1 H)]. (b) ^{1}H NMR spectrum (D_2O) of the ethyl acetate extract of acidified (to pH 2.5) *E. coli aroE*/pKD136 cell lysate which had been exposed to air during incubation with DHS for 20 h at 24 °C. Resonances associated with residual DHS are evident, as are the resonances of product β-ketoadipate: δ 2.66 (t, J = 6 Hz, 2 H), 2.95 (t, J = 6 Hz, 2 H). The remaining protons of β-ketoadipate exchanged with D_2O during ^{1}H NMR sample preparation.

E. coli aroE/pKD136 cell lysate was then incubated with DHS in the presence of oxygen (Figure 1b). Although nearly all of the DHS was consumed, catechol and protocatechuate were barely detectable. One metabolite could be clearly discerned and was

(3) The ^{1}H NMR, ^{13}C NMR, and CI mass spectra of authentic samples were identical to the spectroscopic data collected for catechol, protocatechuate, and β-ketoadipate isolated from culture supernatants and cell lysates.

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determined to be β-ketoadipate. The implied operation of ortho cleavage[6] is the first report of this degradative pathway in *E. coli*.[7] Equivalents of D-glucose lost from the common pathway may thus be converted into β-ketoadipate, which is then further metabolized. Diminished in vitro and in vivo availability of oxygen may limit enzyme-catalyzed reaction of catechol with oxygen, thereby allowing catechol to accumulate.

The conditions which induce DHS dehydratase and protocatechuate decarboxylase in *E. coli* may not be directly applicable to other microbes. Nonetheless, any induction could have a deleterious effect on the percentage of D-glucose converted into desired product. This is of obvious concern in biocatalytic syntheses of L-phenylalanine,[8] L-tryptophan,[9] and indigo,[10] which generally attempt to increase the number of D-glucose equivalents channeled into the common pathway.

As a biocatalytic route to catechol, the induced pathway could be an alternative to the high temperatures, caustic solutions, metals, and peroxides used to chemically produce catechol.[1] An additional attractive feature of the discovered biocatalytic conversion is that D-glucose can be readily obtained from corn starch.[11] This contrasts with all other chemical[1] and biocatalytic syntheses[12] of catechol which utilize starting materials derived from nonrenewable fossil fuels.

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Are Relative Bond Energies a Measure of Radical Stabilization Energies?[1]

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It is a common perception that relative bond dissociation energies (BDE) are a measure of radical stabilization energies (RSE). This idea, which was proposed by O'Neal and Benson,[2] has been questioned by Rüchardt[3] and more recently by Nicholas and Arnold.[4] These authors pointed out that ΔBDE values can be rationalized, alternatively, by considering the effect of substitution on the intrinsic stability of the molecule with its intact bond; i.e., a decrease in ΔBDE may be the result of *destabilization* of the molecule rather than *stabilization* of the radical. Nicholas and Arnold[4] further noted that the ΔBDE values obtained depended on the nature of the R–X bonds that were compared and that ΔBDE was a measure of RSE only for bonds with no net dipole moment.

Table I. Bond Dissociation Energy Data for Some Para-Substituted Benzyl Bromides[a]

X	ΔBDE(X-$C_6H_4CH_2$-Br)[b]
4-Me	0.34, 0.17
4-*t*-Bu	−0.34, 0.40
H	(0.0)[c]
4-F	−0.52
4-Br	−1.94, −1.74
4-CF_3	−2.81
4-CN	−5.50, −4.52,[d] −4.07, −5.84

[a] Measured by photoacoustic calorimetry in triethylsilane/benzene (3:1 v/v). [b] Values represent the results of individual determinations. Each determination consists of at least eight pairs of measurements. [c] All values were determined relative to benzyl bromide (BDE = 59.4 kcal mol^{-1}, ref 12c). [d] The photoacoustic constant (α) was measured from a plot of the signal amplitude versus the laser dose (see ref 8a).

While the limitations of this definition have been addressed, the concept, nevertheless, continues to pervade the recent literature. For example, ΔBDE(O–H) values for a large number of substituted phenols in solution recently have been determined experimentally.[5-7] Bordwell and his co-workers attributed the bond energy differences to changes in the RSE, based on the assumption that the "effects of remote substituents on the ground state of parent molecules will be small compared to that on the radicals".[7] In this work we present evidence that leads us to the conclusion that the effect of remote substituents on the ground state of the parent molecules is important and, in some cases, may indeed be greater than that on the radicals.

We have used photoacoustic calorimetry to determine ΔBDE values for a number of para-substituted benzyl bromides (Table I). This technique is described in detail elsewhere.[8] Briefly, argon-saturated solutions of the benzyl bromide and di-*tert*-butyl peroxide (1–12% v/v) in triethylsilane/benzene (3:1 v/v) were flowed through a standard flow cell and irradiated using a pulsed nitrogen laser (337.1 nm, 84.8 kcal mol^{-1}, eqs 1–3). The net heat evolved from the overall photoreaction (eq 4) produced a shock wave that was detected using a piezoelectric transducer (microphone) that was in contact with the cell. The intensity of the shock wave is proportional to $\Delta H°_4$ when reactions 2 and 3 have lifetimes of <60 ns.[5,9] Using a value of k_2 from the literature[10] and values for k_3 measured by laser flash photolysis ($k_3 > 10^8$ M^{-1} s^{-1}), we were able to determine the concentration range for each reactant that satisfied this time constraint.

$$t\text{-BuO-OBu-}t \xrightarrow{h\nu} 2t\text{-BuO}^{\bullet} \quad (1)$$

$$2t\text{-BuO}^{\bullet} + 2Et_3SiH \rightarrow 2t\text{-BuOH} + 2Et_3Si^{\bullet} \quad (2)$$

$$2Et_3Si^{\bullet} + 2p\text{-X-}C_6H_4CH_2Br \rightarrow 2Et_3SiBr + 2p\text{-X-}C_6H_4CH_2^{\bullet} \quad (3)$$

$$t\text{-BuO-OBu-}t + 2Et_3SiH + 2p\text{-X-}C_6H_4CH_2Br \rightarrow 2t\text{-BuOH} + 2Et_3SiBr + 2p\text{-X-}C_6H_4CH_2^{\bullet} \quad (4)$$

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(9) (a) The rate constant for reaction of $PhCH_2^{\bullet}$ with Bu_3SnH is only ca. 4×10^4 M^{-1} s^{-1} (Franz, J. A.; Suleman, N. K.; Alnajjar, M. S. *J. Org. Chem.* **1986**, *51*, 19). Since the Sn–H bond energy is ≈16 kcal mol^{-1} weaker than the Si–H bond, the reaction of the benzyl radicals with Et_3SiH is expected to be too slow to contribute to the detected acoustic signals. (b) Under the experimental conditions the bromides do not absorb the incident light so the amplitude of the acoustic wave does not depend on the concentration of the bromide. (c) The Φ value of 0.85 has been reported in the literature (Burkey, T. J.; Majewski, M.; Griller, D. *J. Am. Chem. Soc.* **1986**, *108*, 2218. Kanabus-Kaminska, J. M.; Hawari, J. A.; Griller, D. *J. Am. Chem. Soc.* **1987**, *109*, 5267).

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