Thermodynamic and Quantum Chemical Study of the Conversion of Chorismate to (Pyruvate + 4-Hydroxybenzoate)

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A thermodynamic investigation of the conversion of chorismate^{2-}(aq) to {pyruvate$^-$(aq) + 4-hydroxybenzoate$^-$(aq)} has been performed by using microcalorimetry and high-performance liquid chromatography. The study used a genetically engineered sample of chorismate lyase that was prepared with the *Escherichia coli ubiC* gene. The calorimetric measurements led to a standard molar enthalpy change $\Delta_r H^\circ_m = -(144 \pm 7)$ kJ mol^{-1} for this reaction at the temperature $T = 298.15$ K and ionic strength $I_m = 0$. An estimated value of the standard molar entropy change $\Delta_r S^\circ_m = 222$ J K^{-1} mol^{-1} for the above reaction was used together with the experimental value of $\Delta_r H^\circ_m$ to obtain a standard molar Gibbs free energy change $\Delta_r G^\circ_m \approx -210$ kJ mol^{-1} and an equilibrium constant $K \approx 10^{37}$ for the conversion of chorismate^{2-}(aq) to {pyruvate$^-$(aq) + 4-hydroxybenzoate$^-$(aq)} at $T = 298.15$ K and $I_m = 0$. Quantum mechanics (*Gaussian 94* with a B3LYP functional and a 6-31G* basis set) was used to calculate values of absolute energies for the neutral and ionic species pertinent to this reaction both in the gas phase and in aqueous solution. The bond angles and bond lengths in pyruvic acid and 4-hydroxybenzoic acid and their monoanions were also obtained along with values of thermodynamic reaction quantities. The effects of water solvation and solvent polarization were accounted for by using both a polarizable continuum model (PCM) and a self-consistent isodensity polarizable continuum model (SCI-PCM). The calculated value of $\Delta_r H^\circ_m$ for the conversion of chorismate^{2-}(aq) to {pyruvate$^-$(aq) + 4-hydroxybenzoate$^-$(aq)} at $T = 298.15$ K was -154 kJ mol^{-1} with the PCM model and -178 kJ mol^{-1} with the SCI-PCM model. The relatively large discordance in the SCI-PCM calculation may arise from the ill-defined cavity size, which is derived from the solute charge distribution isosurface. However, the PCM model, which employs a parametrized cavity radius, yields a result that can be considered to be in agreement with experiment.

1. Introduction

Chorismate serves as a significant branch point in the metabolic pathway of microorganisms.[1] Specifically, there are five separate enzymes that convert chorismate to prephenate, anthranilate, 4-aminobenzoate, isochorismate, and 4-hydroxybenzoate. We have recently carried out a thermodynamic investigation[2] of the conversion of chorismate to prephenate in which an accurate value for the standard molar enthalpy of this reaction was obtained. This study was complemented by a series of quantum mechanical calculations, which led to values of thermodynamic quantities for the reaction both in the gas phase and in aqueous solution. Also, while it was not possible to measure an equilibrium constant, we were able to use an estimated standard molar entropy change in conjunction with the measured standard molar enthalpy of reaction to obtain an approximate value for the equilibrium constant. An additional branch point reaction in this pathway has been studied by Liu[1,3] who reported an apparent equilibrium constant for the conversion of chorismate to isochorismate. Since accurate thermodynamic results are a prerequisite for the quantitative analysis of energy transformations in living systems,[4] we have undertaken this study, which has as its primary aim an improved knowledge of the thermodynamics of the biochemical reaction:

$$\text{chorismate(aq)} = \text{4-hydroxybenzoate(aq)} + \text{pyruvate(aq)} \quad (1)$$

This is the branch point in the chorismate pathway that leads to the formation of ubiquinone (coenzyme Q). The chemical changes in this reaction are primarily the cleavage of the 2-alkylpropenoic acid side chain from the chorismate and a resonance stabilization of the product 4-hydroxybenzoate (see Figure 1). This is in contrast to the previously studied[2] Claisen rearrangement of chorismate to prephenate (also see Figure 1). Reaction 1 is catalyzed by chorismate lyase,[5,6] which is encoded by the *E. Coli ubiC* gene. While the molecular biology of this gene has been recently investigated by several workers,[7−9] many aspects of the enzymology and of the reaction itself remain relatively unstudied.

In our previous investigation,[2] reasonable agreement was found between the calculated and measured values of the standard molar enthalpy change $\Delta_r H^\circ_m$ for the conversion of chorismate to prephenate. This agreement between theory and experiment, while it was very encouraging, may have been fortuitous due to the symmetry of the reaction and a cancelation of hydration effects. Therefore, it was deemed interesting to attempt calculations on reaction 1, which presents a much more

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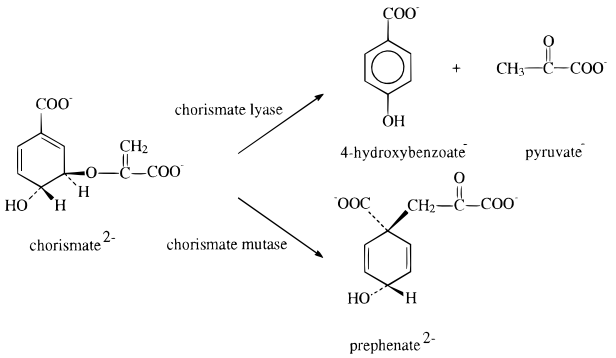

Figure 1. Conversion of chorismate^{2-} to (pyruvate$^-$ + 4-hydroxybenzoate$^-$) and of chorismate^{2-} to prephenate^{2-}.

challenging problem in that the effects of hydration are much less likely to cancel. In addition to its inherent biochemical importance, the conversion of chorismate to 4-hydroxybenzoate is also of relevance to biotechnology where there is significant interest in the use of the chorismate pathways for the manufacture of ubiquinone, aromatic amino acids, enterobactin, and other useful products.[10,11]

2. Methods

2.1. Materials. Relevant information on the substances used in this study is given in Table S1 contained in the Supporting Information for this paper.[12] The sample of chorismate used in the study was characterized in an earlier study.[2] The identified impurities in this sample and their respective mass fractions w are water, 0.102; diethyl ether, 0.0225; 4-hydroxybenzoate, 0.0043; prephenate, 0.0024; and β-phenylpyruvate, 0.0044. The sample also contained additional unidentified impurities having a combined mass fraction of ≈ 0.015. Thus, the combined mass fraction of all impurities (including water) in the sample is 0.151. This mass fraction was used in all subsequent calculations that involved the amount of chorismate.

The chorismate lyase protein was expressed in *E. coli* (DH5α) cells that carried a proprietary plasmid containing the *ubiC* gene. The bacterial cell cultures were grown in a batch culture solution in Terrific Broth medium[13] at 30 °C; ampicillin (0.1 g dm^{-3}) was added to the culture to maintain the plasmid. After 14 h, the cells were harvested and subsequently lysed by a freeze−thaw procedure carried out in a buffer solution {3-(*N*-morpholino)propanesulfonic acid (MOPS) (0.020 mol dm^{-3}) + sodium acetate (0.008 mol dm^{-3}) + dithiothreitol (DTT) (0.004 mol dm^{-3}) + ethylenediaminetetraacetic acid (EDTA) (0.010 mol dm^{-3}) + phenylmethylsulfonyl fluoride (0.001 mol dm^{-3}), adjusted to pH = 7.8 with solid tris(hydroxymethyl)aminomethane (Tris), followed by the addition of lysozyme (0.2 g dm^{-3})}. After 1 h at 4 °C, MgSO4 (0.010 mol dm^{-3}) and DNase (0.002 g dm^{-3}) were added to the cells in the aforementioned buffer. Lysis was followed by centrifugation at 29000g_n (g_n = 9.80665 m s^{-2}) for 1 h at 4 °C. The supernatant was then pumped onto a Pharmacia DEAE-Fast Flow Sepharose anion exchange column that had been previously equilibrated with the buffer {MOPS (0.050 mol dm^{-3}) + DTT (0.004 mol dm^{-3}), adjusted to pH = 8.5 with solid Tris}. Even though the chorismate lyase did not bind to the column and was eluted in the wash-through, a significant purification did occur through the binding of contaminants in the column.

The second purification step for the chorismate lyase involved hydrophobic interaction chromatography. First, the chorismate lyase fractions were diluted to lower the concentration of DTT to 0.001 mol dm^{-3} and then solid $(NH_4)_2SO_4$ was added so that its concentration in the solution was 1.0 mol dm^{-3}. This solution was then pumped onto a phenyl Sepharose column (diameter = 2.5 cm, length = 10 cm, volume = 0.050 dm^{-3}) that had been equilibrated with $(NH_4)_2SO_4$ (1.0 mol dm^{-3}). A gradient {flow rate = 5.0 cm^3 min^{-1}, $(NH_4)_2SO_4$ at the concentration c = 1.0 mol dm^{-3} at time t = 0, c = 1.0 mol dm^{-3} at t = 10 min, and c = 0 at t = 30 min} was then run, and the fractions that represented the center of the peak of chorismate lyase activity were pooled. During this purification procedure, the activity of the chorismate lyase was measured by using a coupled assay adapted from Siebert et al.[9] The mass fraction of protein in the solution was ≈0.01, as determined by using a Bio-Rad total protein assay. The activity of the enzyme was $\approx 3 \times 10^{-5}$ mol g^{-1} s^{-1} under the experimental conditions used in this study. Just prior to use, the chorismate lyase was passed through a desalting column with the respective buffers used in the equilibrium and calorimetric experiments.

2.2. Chromatography. The mixture of (pyruvate + chorismate + 4-hydroxybenzoate) was separated with a Hewlett-Packard 1050 HPLC equipped with a UV detector set at the wavelength λ = 215 nm and a Bio-Rad Aminex HPX-87H column (7.8 mm i.d. × 100 mm long) thermostated at the temperature T = 313 K. The mobile phase consisted of (I) H_2SO_4 (concentration c = 0.005 mol dm^{-3}) and (II) acetonitrile. The following gradient of these two mobile phases was formed: volume fraction ϕ(I) = 0.96 and ϕ(II) = 0.04 at time t = 0; ϕ(I) = 0.40 and ϕ(II) = 0.60 at t = 22 min. The flow rate was 0.8 cm^3 min^{-1}. Approximate retention times were pyruvate, 3.7 min; chorismate = 7.4 min; and 4-hydroxybenzoate, 11.7 min.

2.3. Equilibrium Measurements. The aim in the equilibrium measurements was to measure the extent of the reaction with equilibrium being approached from both directions of reaction. The solution used for the forward direction of reaction contained chorismate at a molality m = 0.0036 mol kg^{-1} in phosphate buffer {K_2HPO_4 (m = 0.098 mol kg^{-1}) adjusted to pH = 7.0 with H_3PO_4} containing NaCl (m = 0.11 mol kg^{-1}). The solution used for the reverse direction of reaction contained pyruvate (m = 0.0049 mol kg^{-1}) and 4-hydroxybenzoate (m = 0.0045 mol kg^{-1}) in the same (phosphate + NaCl) buffer. Chorismate lyase was then added to these two solutions so that the mass fraction of the enzyme in these solutions was $\approx 4 \times 10^{-4}$. These solutions were placed in 20 cm^3 Teflon-capped glass bottles and gently shaken at ≈50 rpm in a water bath thermostated at T = 298.15 K. Following equilibration for 24 h, the molalities of chorismate, pyruvate, and 4-hydroxybenzoate in these solutions were measured by using the chromatographic procedure described above. The final pHs of the forward and reverse reaction mixtures were, respectively, 6.79 and 6.81.

2.4. Calorimetry. Three heat-conduction microcalorimeters were used for the enthalpy of reaction measurements. They were calibrated electrically with a high stability dc power supply, calibrated digital voltmeter, standard resistor, and time-interval counter. Descriptions of the microcalorimeters and their performance characteristics, the data-acquisition system, and the computer programs used to treat the results have been given by Steckler et al.[14,15] The data acquisition system has recently undergone significant modernization. The voltages of the thermopiles of the microcalorimeters are now measured with Hewlett-Packard model 34420A Nanovolt Meters. These voltages are then recorded on a microcomputer with a data acquisition program written in Hewlett-Packard HP-VEE. The integration of the areas of the thermograms is done by using a code written in C++.

The sample vessels, fabricated from high-density polyethylene, contained two compartments that held ≈0.55 and ≈0.40 cm^3 of solution. The substrate solution (placed in the 0.55 cm^3 compartment) contained chorismate dissolved in a phosphate buffer containing NaCl. The enzyme solution consisted of the chorismate lyase dissolved in the same buffer containing NaCl that was used for the substrate solution. The enzyme solution was placed in the 0.40 cm^3 compartment. The vessels and their contents were allowed to equilibrate in the microcalorimeters for ≈55 min before the enzyme and substrate solutions were mixed. Following reaction times of 41−72 min, the reaction vessels were removed from the microcalorimeters and their contents were promptly analyzed by using the HPLC procedure described above. In this way, the mole fraction of unreacted chorismate was found to vary from 0.008 to 0.025. These mole fractions were used as corrections in the calculation of the molar enthalpies of reaction. The "blank" enthalpy change for the mixing of the substrate solution with the buffer was −(0.7 ± 1.2) mJ; for the mixing of the enzyme solution with the buffer the enthalpy change was (1.4 ± 1.3) mJ. These "blank" enthalpies of mixing were applied as corrections to the measured calorimetric enthalpies $\Delta_r H_m$(cal), which were in the range −1.4 to −1.9 J. In addition to corrections for the impurities in the chorismate sample, it had also been found[2] that 2.4% of the chorismate had spontaneously converted to a mixture of prephenate, β-phenylpyruvate, and 4-hydroxybenzoate after 65 min in both buffers at $T = 298.15$ K. Thus, a correction for this spontaneous decomposition was also applied in the calculation of the molar enthalpies of reaction.

2.5. Quantum Mechanical Calculations. Ab initio calculations were carried out with *Gaussian 94*.[16] All structures were fully optimized with a B3LYP functional and a 6-31G* basis set. Frequency calculations were performed to confirm the nature of the minima and to obtain the zero-point vibrational energies. Thermodynamic properties were computed with standard statistical mechanics methods. Solvation effects were modeled by using a polarizable continuum model (PCM)[17,18] and a self-consistent isodensity polarizable continuum model (SCI-PCM).[19,20] Solvation energies were obtained by single point calculations performed on gas-phase optimized structures. A relative permittivity $\epsilon_r = 81.0$ and an isodensity surface of 0.0004 electrons $Å^{-3}$ were employed for the SCI-PCM calculation. For the PCM calculation, the same relative permittivity and Merz−Kollman atomic radii[21] were employed; the solute cavity was constructed from intersecting spheres. Clearly this continuum model does not treat the explicit inclusion of water molecules. Nevertheless, we shall refer to the results of these calculations by using the terms "hydration" or "solvation."

3. Results and Discussion

3.1. Thermodynamic Formalism. The apparent equilibrium constant[22] corresponding to the overall biochemical reaction 1 is

$$K' = m(\text{pyruvate}) \times m(\text{4-hydroxybenzoate})/\{m(\text{chorismate}) \times m^\circ\} \quad (2)$$

The molalities m in eq 2 are the total molalities of the various charged and uncharged species that are formed from the dissociation of the various substances in solution. However, it will be seen that, under the experimental conditions used in this study, there is a predominant species present in each case. In the discussion of the thermodynamics of this reaction it is also useful to introduce the following reference reaction:

$$\text{chorismate}^{2-}(\text{aq}) = \text{pyruvate}^{-}(\text{aq}) + \text{4-hydroxybenzoate}^{-}(\text{aq}) \quad (3)$$

The equilibrium constant for this reaction is

$$K = m(\text{pyruvate}^{-}) \times m(\text{4-hydroxybenzoate}^{-})/\{m(\text{chorismate}^{2-}) \times m^\circ\} \quad (4)$$

Throughout this study, the standard state is taken to be the hypothetical ideal solution of unit molality ($m^\circ = 1$ mol kg^{-1}).

Information on the state of ionization of the reactants and products and of the other solutes in solution is needed to relate the experimental results for the biochemical reaction 1 to thermodynamic quantities for the reference reaction 2. At $T = 298.15$ K and ionic strength $I_m = 0$, the pK of pyruvic acid is 2.49 and the pK's of 4-hydroxybenzoic acid are 4.58 and 9.46.[23] On the basis of the known pK's of other substances that are structurally similar to chorismic acid, its pK's were previously judged[2] to be <4.5. This estimate was also found to be consistent with the approximate results obtained from a potentiometric titration that was performed[2] on chorismic acid. These aforementioned pK's and an estimated pK of 4.5 for chorismic acid were used in all subsequent calculations. Since the calorimetric experiments were done at 6.92 ≤ pH ≤ 7.04, the difference |pH − pK| > 2.4 and the predominant species (mole fraction x >0.996) in the calorimetric experiments are chorismate^{2-}, pyruvate^{-}, and 4-hydroxybenzoate^{-}.

3.2. Results of Equilibrium and Calorimetric Experiments. The result of the equilibrium measurements was that no measurable amount of chorismate was present in either the forward or reverse reaction mixtures. Thus, it was not possible to measure an equilibrium constant. On the basis of the lower limit of the amount of chorismate detectable with our chromatographic procedure, we can state that $K'(T = 298.15$ K, pH $= 6.8) > 10$ for biochemical reaction 1. The aforementioned inequality also holds for the equilibrium constant for the reference reaction 3 and is consistent with the earlier report by Siebert et al.[9] that a reverse reaction could not be observed.

The results of the calorimetry experiments are given in Table 1. This table also contains values of the ionic strength I_m and of $\Delta_r H_m^\circ$, the standard molar enthalpy change for the reference reaction 2 at $I_m = 0$. The equilibrium model used to calculate $\Delta_r H_m^\circ$ from the measured values of $\Delta_r H$(cal) has been described previously.[24] The computations have been modified recently so that it now utilizes the Mathematica[25] computer code of Alberty and Krambeck[26] to solve the simultaneous nonlinear equations that describe the various equilibria. This Mathematica code has been extended so as to include corrections for nonideality and so that the calculations are made self-consistent[24] in regard to the ionic strength. The nonideality corrections are based on the extended Debye−Hückel equation in which the "ion-size" parameter has been set at 1.6 $kg^{1/2}$ $mol^{-1/2}$. Since the change in binding of hydrogen $\Delta_r N(H^+)$ for reaction 1 was relatively small (≈0.01), the buffer protonation corrections[27] to the calorimetric enthalpies were negligible (≈0.040 kJ mol^{-1}). The constant difference $\{\Delta_r H_m(\text{cal}) - \Delta_r H_m^\circ\} = -1.0$ kJ mol^{-1} is almost entirely due to the adjustment of the ionic strength from $I_m = 0.38$ mol kg^{-1} to $I_m = 0$.

From Table 1 we obtain a mean value of $\langle \Delta_r H_m^\circ \rangle = -(144.1 \pm 2.6)$ kJ mol^{-1}. However, this uncertainty indicates only the random errors inherent in the measurements and does not reflect the possible systematic errors that are mostly attributable to impurities in the chorismate and its spontaneous decomposition to prephenate in solution. Thus, the error analysis is essentially

TABLE 1: Results of Calorimetric Measurements for Biochemical Reaction 1 in Phosphate Buffer at $T = 298.15$ K[a]

	molality/mol kg^{-1}							
expt	$m(K_2HPO_4)$	$m(H_3PO_4)$	m(NaCl)	m(chorismate)	pH	I_m/mol kg^{-1}	Δ_rH_m(cal)/kJ mol^{-1}	$\Delta_rH_m^\circ$/kJ mol^{-1}
1	0.1021	0.010 74	0.099 23	0.012 31	6.95	0.38	−143.1	−142.1
2	0.1021	0.010 74	0.099 23	0.012 61	6.95	0.38	−140.7	−139.7
3	0.1021	0.010 74	0.099 23	0.012 95	6.95	0.38	−140.2	−139.2
4	0.1021	0.010 73	0.099 21	0.016 41	6.92	0.38	−147.8	−146.8
5	0.1021	0.010 73	0.099 21	0.016 42	6.92	0.38	−145.9	−144.9
6	0.1021	0.010 74	0.099 23	0.012 76	7.04	0.38	−144.5	−143.5
7	0.1021	0.010 74	0.099 24	0.011 14	7.04	0.38	−148.9	−147.9
8	0.1021	0.010 74	0.099 23	0.011 51	7.04	0.38	−150.0	−149.0

$\langle\Delta_rH_m^\circ\rangle = -144.1 \pm 2.6$ kJ mol^{-1}

[a] The molalities m are those obtained after mixing of the enzyme and substrate solutions and prior to any reaction. All molalities are equal to the sums of the molalities of the indicated substances in their various ionic forms. Δ_rH_m(cal) is the calorimetrically determined molar enthalpy of reaction 1. $\Delta_rH_m^\circ$ is the standard molar enthalpy at $I_m = 0$ for the reference reaction 2. The statistical uncertainty of ±2.6 kJ mol^{-1} in the average value of $\Delta_rH_m^\circ$ is equal to two estimated standard deviations of the mean; the final estimate of error is ±6.6 kJ mol^{-1} (see Results and Discussion). The values of the ionic strength I_m and $\Delta_rH_m^\circ$ were calculated. The mass fraction w of the chorismate lyase in solution was ≈0.0023.

the same as in our previous study[2] on the thermodynamics of the conversion of chorismate to prephenate. Accordingly, we judge that reasonable estimates of the standard uncertainties[28] due to these possible systematic errors in Δ_rH_m(cal) are heat measurements, $0.003\Delta_rH_m$(cal); incomplete reaction and possible side reactions, $0.010\Delta_rH_m$(cal); moisture content in the chorismate, $0.005\Delta_rH_m$(cal); and impurities in the chorismate, $0.017\Delta_rH_m$(cal). The possible error attributable to the ionic strength correction was evaluated by perturbing the "ion-size" parameter used in the activity coefficient model by ±0.3 kg$^{1/2}$ mol$^{-1/2}$. In this way, it was found that the effect on the calculated value of $\Delta_rH_m^\circ$ was negligible (<0.1 kJ mol^{-1}). These estimates of the standard uncertainties due to possible systematic error are combined in quadrature together with the statistical uncertainty in the measured value of $\Delta_rH_m^\circ$, expressed as one estimated standard deviation of the mean, to obtain a combined standard uncertainty.[28] This combined standard uncertainty is then multiplied by 2 to arrive at the final result: $\Delta_rH_m^\circ = -(144 \pm 7)$ kJ mol^{-1}.

3.3. Benson Estimates. We are unaware of any thermochemical pathways that permit a comparison or check on our experimental result for $\Delta_rH_m^\circ$ for reaction 2. However, we are able to use the Benson group additivity method[29,30] to estimate values of the standard molar enthalpies of formation $\Delta_fH_m^\circ$ and standard molar entropies S_m° of chorismic, pyruvic, and 4-hydroxybenzoic acids in the gas phase. These enthalpies of formation and entropies are then combined to obtain estimates of $\Delta_rH_m^\circ$ and $\Delta_rS_m^\circ$ for the reaction

chorismic acid(g) = pyruvic acid(g) + 4-hydroxybenzoic acid(g) (5)

Thus, by using the Benson group values tabulated by Domalski and Hearing,[31] we obtain $\Delta_rH_m^\circ = -123$ kJ mol^{-1} and $\Delta_rS_m^\circ = 257$ J mol^{-1} K^{-1} for reaction 5 at $T = 298.15$ K and pressure $P = 0.101\,325$ MPa. In the Benson calculation, the major contribution (−92 kJ mol^{-1}) to $\Delta_rH_m^\circ$ for reaction 5 comes from the cleavage of the 2-oxapropenoic acid side chain from chorismic acid to form pyruvic acid. The balance (−31 kJ mol^{-1}) comes largely from resonance stabilization of the six-membered ring. In the calculation of $\Delta_rS_m^\circ$ with the Benson method,[31] it was necessary to estimate the values of the entropies for the $C_B-CO(C_B)_2$, $CO-(O)(C_B)$, CO−(O)−(CO), and CO−(C)(CO) groups. We note that any comparison with or application of these estimated values to reaction 2, which pertains to the aqueous phase, completely neglects the hydration and ionization of the reactants. Thus, the reasonable accord of the estimated value of $\Delta_rH_m^\circ = -123$ kJ mol^{-1} for reaction 5 with the experimental result of $\Delta_rH_m^\circ = -(144 \pm 7)$ kJ mol^{-1} for reaction 2 may be fortuitous.

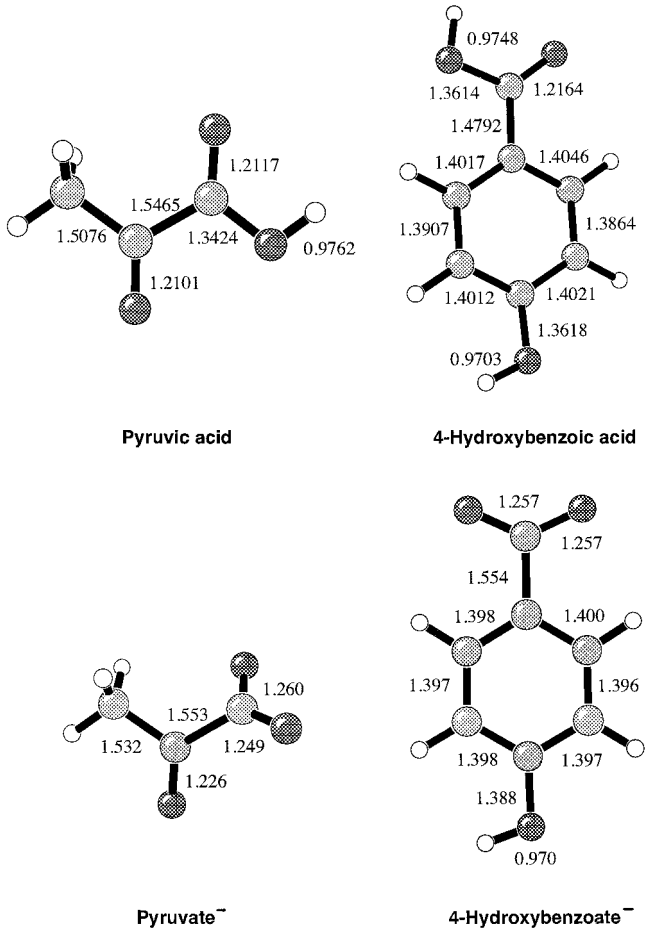

Figure 2. Structures of pyruvic acid[0], pyruvate$^-$, 4-hydroxybenzoic acid[0], and 4-hydroxybenzoate$^-$ as obtained from quantum mechanical calculations. The distances are in angstroms. The Cartesian coordinates are given in Table S2 contained in the Supporting Information for this paper.

3.4. Results of Quantum Mechanical Calculations. The optimized geometries of pyruvic acid, 4-hydroxybenzoic acid, and their monoanions are shown in Figure 2. Table 2 contains calculated absolute energies and thermodynamic properties for the chorismic acid, pyruvic acid, and 4-hydroxybenzoic acid species. The bond angles, bond lengths, and energies of the

TABLE 2: Absolute Energies (Hartree Molecule^{-1}) and Entropies (J K^{-1} mol^{-1}) for the Chorismic Acid, Pyruvic Acid, and 4-Hydroxybenzoic Acid Species Calculated at the B3LYP/6-31G* Level[a,b]

species	E(SCF)	E(SCF) + E(ZP)	H	G	S	E(SCI-PCM)	E(PCM)
chorismic acid^0, pseudodiequatorial	−838.370 52[c]	−838.176 07[c]	−838.160 22[c]	−838.219 64[c]	523.1[c]	−838.388 00	−838.408 69
chorismate^{2-}, pseudodiequatorial	−837.185 76[c]	−837.017 71[c]	−837.002 64[c]	−837.060 41[c]	508.8[c]	−837.422 00	−837.505 52
chorismic acid^0, pseudodiaxial	−838.366 66[c]	−838.172 03[c]	−838.155 93[c]	−838.217 33[c]	540.6[c]	−838.385 44	−838.404 57
chorismate^{2-}, pseudodiaxial	−837.166 13[c]	−836.998 13[c]	−836.982 56[c]	−837.041 66[c]	520.5[c]	−837.407 67	−837.495 41
pyruvic acid^0	−342.395 02	−342.323 62	−342.31632	−342.354 30	334.5	−342.406 25	−342.414 67
pyruvate$^-$	−341.836 52	−341.778 50	−341.771 23	−341.809 38	335.9	−341.927 04	−341.962 20
4-hydroxybenzoic acid^0	−496.040 41	−495.920 40	−495.911 22	−495.953 75	374.5	−496.053 84	−496.060 47
4-hydroxybenzoate$^-$	−495.470 09	−495.355 79	−495.354 85	−495.397 25	373.4	−495.562 56	−495.602 14

[a] E(SCF) is the self-consistent field energy; E(ZP) is the zero-point energy; H, G, and S are respectively the enthalpy, Gibbs free energy, and entropy; 1 hartree = 4.3597482 × 10^{-18} J; 1 hartree molecule^{-1} = 2625.50 kJ mol^{-1}. The solvation energy is equal to E(SCF) plus the cavity contribution, i.e., either E(SCI-PCM) or E(PCM). [b] The quantities E(SCF), E(SCI-PCM), and E(PCM) refer to T = 0; E(SCF) + E(ZP) refers to T = 0 and P = 0.101 325 MPa; H, G, and S refer to T = 298.15 K and P = 0.101 325 MPa. [c] This value is from Kast et al.[2]

TABLE 3: Thermodynamic Reaction Quantities Calculated Using Quantum Mechanics[a-d]

condition	$\Delta_r H^\circ_m$/kJ mol^{-1}	$\Delta_r S^\circ_m$/J K^{-1} mol^{-1}
Chorismic Acid^0 (Pseudodiequatorial) = Pyruvic Acid^0 + 4-Hydroxybenzoic Acid^0		
gas phase	−177	186
aqueous solution (SCI-PCM)	−189	
aqueous solution (PCM)	−175	
Chorismic Acid^0 (Pseudodiaxial) = Pyruvic Acid^0 + 4-Hydroxybenzoic Acid^0		
gas phase	−188	168
aqueous solution (SCI-PCM)	−196	
aqueous solution (PCM)	−185	
Chorismate^{2-}(Pseudodiequatorial) = Pyruvate$^-$ + 4-Hydroxybenzoate$^-$		
gas phase	−324	201
aqueous solution (SCI-PCM)	−178	
aqueous solution (PCM)	−154	
Chorismate^{2-}(Pseudodiaxial) = Pyruvate$^-$ + 4-Hydroxybenzoate$^-$		
gas phase	−377	189
aqueous solution (SCI-PCM)	−215	
aqueous solution (PCM)	−181	

[a] $\Delta_r H^\circ_m$ and $\Delta_r S^\circ_m$ are respectively the standard molar enthalpy and standard molar entropy of reaction at T = 298.15 and P = 0.101 325 MPa. [b] $\Delta_r H^\circ_m$ for the reaction in solution is approximated by using either E(SCI-PCM) or E(PCM). [c] Since the pseudodiequatorial form of chorismate^{2-} is 49 kJ mol^{-1} more stable than the pseudodiaxial form, results for its reactions are more relevant in comparisons with the experimental results. [d] In the absence of a model that yields the pertinent frequencies of the species in solution, we are unable to calculate the partial molar entropies of these species as well values of $\Delta_r S^\circ_m$ for the reactions in solution.

chorismic acid species were obtained previously[2] at the same computational level and are used in the present study.

The calculated thermodynamic quantities for the reactions in the gas phase and in water are given in Table 3. The reaction is highly exothermic for all geometries and phases. There is also, in all cases, a substantial entropic contribution (≥50 kJ mol^{-1}) to the standard molar Gibbs free energy of reaction $\Delta_r G^\circ_m$. Both the SCI-PCM and PCM models indicate that, for the reactions involving the neutral species, the changes in $\Delta_r H^\circ_m$ due to inclusion of hydration are not large (≤12 kJ mol^{-1}). However, the effects of hydration are very substantial (≥147 kJ mol^{-1}) for the reactions that involve charged species. Thus, for the reaction

$$\text{chorismate}^{2-}\text{(pseudo diequatorial)(g)} = \text{pyruvate}^-\text{(g)} + \text{4-hydroxybenzoate}^-\text{(g)} \quad (6)$$

the calculated value of $\Delta_r H^\circ_m$ is −324 kJ mol^{-1} at T = 298.15 K and P = 0.101 325 MPa. The inclusion of solvation effects decreases the calculated exothermicity of the reaction by 170 kJ mol^{-1} when the PCM model is used and by 146 kJ mol^{-1} when the SCI-PCM model is used. Thus, $\Delta_r H^\circ_m$ = −154 kJ mol^{-1} for reaction 3 with the PCM model, a result which is in satisfactory agreement with the experimental value −(144 ± 7) kJ mol^{-1}. However, the SCI-PCM model gives $\Delta_r H^\circ_m$ = −178 kJ mol^{-1} for reaction 3 and thus is in discordance by 34 kJ mol^{-1}. The poorer performance of the SCI-PCM model is probably due to the method used to define the size and shape of the solute cavity. In the conversion of chorismate^{2-} to prephenate^{2-}, there is little change in the solute cavity size and this may have led to a fortuitous cancelation of errors in the previously performed[2] study where the calculated value of $\Delta_r H^\circ_m$ was in very good agreement with the experimental result. However, there is a dramatic change of the solute cavity size in the reaction studied herein. Thus, the relatively large discordance in the SCI-PCM calculation may come from the ill-defined cavity size, which is derived from the solute charge distribution isosurface.[32,33] In this case, the PCM model, which employs a parametrized cavity radius, is more suitable for solvation energy calculations. Clearly, better ways to define the molecular shape in the solvation calculation could lead to a significant improvement in the calculation of the hydration effects.

3.5. Estimation of the Equilibrium Constant. It is seen from the above discussion that significantly different values (Δ = 44 kJ mol^{-1}) of $\Delta_r H^\circ_m$ for reaction 5 were obtained from the Benson method and from the quantum mechanical calculations. Additionally, the values of $\Delta_r S^\circ_m$ for this reaction are also significantly different (Δ = 71 J K^{-1} mol^{-1}). Since neither $\Delta_r H^\circ_m$ nor $\Delta_r S^\circ_m$ are experimentally accessible, it is not possible to determine which of the two methods (Benson or quantum mechanics) has provided the better result. Despite these difficulties, we use the average value $\langle \Delta_r S^\circ_m \rangle$ = 222 J K^{-1} mol^{-1} as a crude estimate of $\Delta_r S^\circ_m$ for reaction 3. This estimate of $\Delta_r S^\circ_m$ neglects the effects of both hydration and ionization and is obviously a gross approximation. Combination of this estimate with $\Delta_r H^\circ_m$ = −144 kJ mol^{-1} for reaction 3 leads to $\Delta_r G^\circ_m \approx$ −210 kJ mol^{-1} for this reaction and a corresponding equilibrium constant $K \approx 10^{37}$. If the estimated value of $\Delta_r S^\circ_m$ were in error by 150 J K^{-1} mol^{-1}, K would still be >10^{29}. Thus, for all practical purposes, reaction 3 can be considered to be "irreversible."

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Supporting Information Available: One table containing information on the materials used in this study and a second table giving the Cartesian coordinates of the pyruvic acid, 4-hydroxybenzoic acid, and their monoanions as obtained from the quantum mechanical calculations (3 pages). Ordering information is given on any current masthead page.

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