Conformational and solvation aspects of the chorismate–prephenate rearrangement studied by *ab initio* electronic structure and simulation methods

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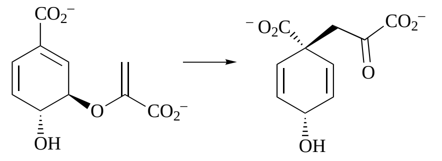
The effect of water on the energetics of the Claisen rearrangement of chorismate to prephenate has been investigated with both a continuum model (PCM) and explicit solvation within a Monte Carlo (MC) free energy perturbation (FEP) treatment. Both models are surprisingly similar in their quantitative predictions. Solvation is shown to increase the conformational flexibility in the reactant by reducing the energy difference between the diequatorial and diaxial structures, and to lead to a considerable reduction in the barrier to the reaction. The estimate of the activation barrier, 22 kcal mol^{-1},† is very close to the experimental value of 24.5 kcal mol^{-1}. The implications of the various possible conformers on kinetic isotope effects are discussed.

Introduction

The structural and energetic aspects of the conversion of chorismate (**1**) to prephenate (**2**) have attracted much experimental and theoretical interest. This reaction occurs both in aqueous solution and also at the active site of the enzyme chorismate mutase.[1] This enzyme is involved in the shikimic acid pathway to give aromatic amino acids in bacteria, fungi and higher plants[2,3] and hence appropriate inhibitors are of interest for their possible role as herbicides and antibiotics.[4,5] In the design of such inhibitors the concept of transition state analogues is frequently employed.[6–9] Thus, insights into the structure and energetics of the transition state involved in this reaction have been sought both experimentally[10–14] and theoretically.[15–21] The calculated transition state structure, obtained at an *ab initio* level, has been discussed by Wiest and Houk,[15] whilst we have quantified the similarity between the transition structure and that of a potent inhibitor of the enzyme catalysed reaction.[16] Experimentally, kinetic isotope effects (KIE) have been measured for the reaction in aqueous solution and in chorismate mutase, in order to gain insight into the transition state structure.[17,18] We here examine important aspects of this reaction, not previously investigated theoretically, namely the conformational energetics of both reactant and transition state, the effect of solvation on these different structures, and the implications of these different structures on the interpretation of the measured KIEs. To study the reaction in an aqueous environment both an *ab initio* continuum model, that does not consider the detailed molecular nature of the solvent–solute interactions, and a classical simulation method that includes explicit water molecules, have been employed. In the latter approach, a classical force field within a Monte Carlo (MC) free energy perturbation (FEP) method has been used to solvate structures along the reaction pathway obtained from *ab initio* electronic structure calculations of the gas phase reaction.



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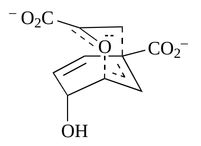

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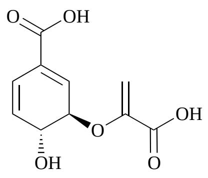

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Computational details

Electronic structure calculations. Stationary points on the gas phase potential energy surfaces of chorismate (**1**), the transition state (**3**) for its conversion to prephenate, and of chorismic acid (**4**), along with the corresponding transition states, were found at the Hartree–Fock level using a 6-31G* basis [6-31G*(HF)]. All structures were characterised as minima or transition states by evaluation of the harmonic frequencies. Electron correlation was considered by carrying out calculations at the MP2 level, using these structures [6-31G*(MP2)//6-31G*(HF)]. Within an electronic structure model we have included the effect of solvation by using the polarisable continuum model (PCM) as developed by Miertus *et al.*[22] The variant we use here (SCIPCM) as implemented in Gaussian 94[23] employs an isodensity surface, determined self-consistently, to describe the solvent cavity. The 0.001e isodensity surface and a relative permittivity of 78.4 were employed. The solvation energies were calculated for the gas phase stationary structures [6-31G*-(PCM)//6-31G*(HF)]. The harmonic frequencies, calculated for the predicted stationary structures, were used to estimate the KIEs from transition state theory within the ideal gas, rigid rotor, harmonic oscillator approximation. The RHF frequencies were used and scaled by the usual factor of 0.89.

Monte Carlo calculations. FEP calculations were carried out employing the gas phase stationary structures and associated atomic charges of chorismate (**1**) and the transition state (**3**), thus allowing the relative solvation free energies of these structures to be obtained. The MC simulations were performed with the BOSS program[24] employing isothermal–isobaric (NPT) ensembles with one solute molecule (with the internal degrees of freedom constrained at the appropriate geometry obtained from the electronic structure calculation) solvated by 740 TIP4P water molecules. The intermolecular potential function

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† 1 cal = 4.184 J.

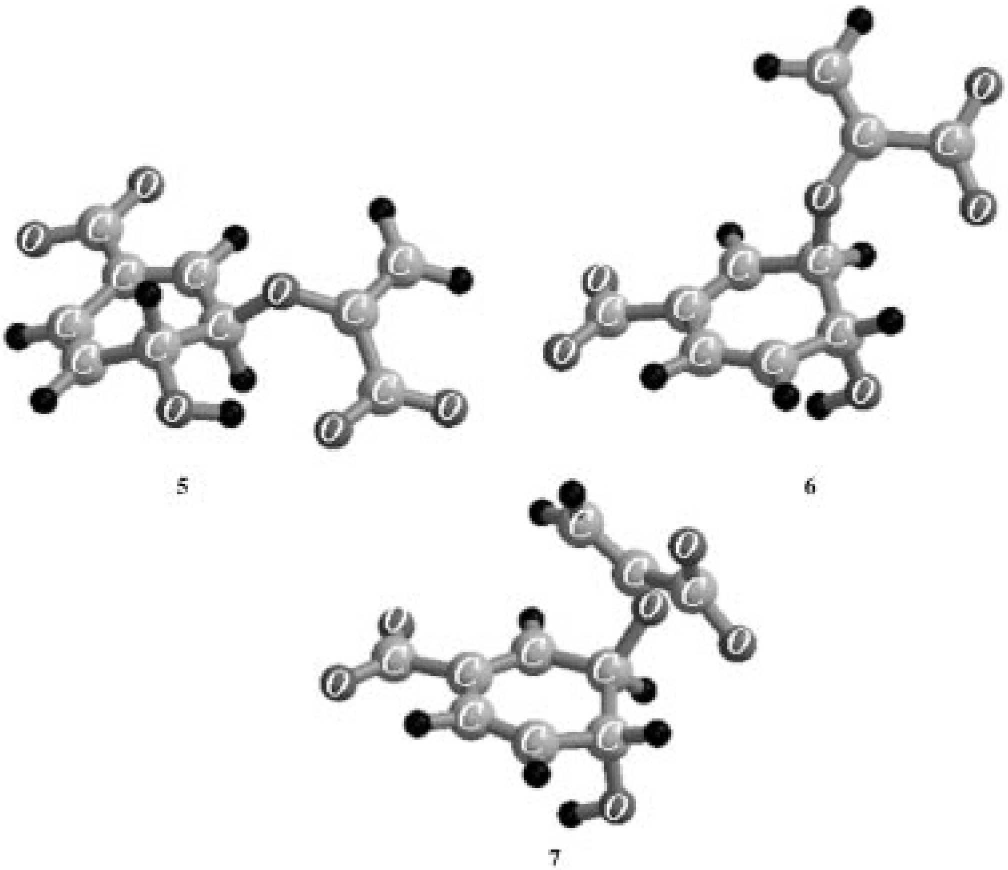


Fig. 1 Reactant structures of chorismate (**1**) comprising the diequatorial (**5**) and two diaxial (**6** and **7**) structures

Table 1 Energies (au) of chorismate, chorismic acid and transition state (TS) structures[a]

	Diequatorial	Diaxial		TS	
	5	**6**	**7**	OHin **3a**	OHout **3b**
Chorismic acid [6-31G*(HF)//6-31G*(HF)]	−833.650 33 (−833.648 98)	−833.642 75 (−833.640 02)	−833.637 65	−833.568 75	−833.566 13
Chorismate [6-31G*(HF)//6-31G*(HF)]	−832.454 91	−832.436 35 (−832.429 33)	−832.426 99	−832.349 80	−832.343 35 (−832.343 82)
Chorismate [6-31G*(MP2)//6-31G*(HF)]	−834.778 70	−834.760 38	−834.752 29	−834.721 35	−834.713 09
Chorismate [6-31G*(PCM)//6-31G*(HF)]	−832.689 89	−832.682 67	−832.673 88	−832.608 48	−832.605 62

[a] The values in parentheses are from ref. 15.

comprised Coulomb and Lennard–Jones terms. The formal charges for the solutes to be used in the MC simulations were determined from the 6-31G*(HF) gas phase wavefunctions *via* a fit of the electrostatic potential surface using the CHELPG[25] method. OPLS all-atom L-J parameters (σ,ε) were taken, with geometric combining rules.[26,27] Simulations were carried out at 25 °C and 1 atm pressure. Equilibration runs were performed for typically 1–2 × 10^6 configurations, followed by data collection for 2–4 × 10^6 configurations. The free energy perturbation (FEP) simulations were carried out in both the forward and backward directions using increments of 0.1 for the coupling parameter. Solute–solvent and solvent–solvent interactions (Coulombic and Lennard–Jones) were given a cut-off of 12 Å (half the box length), as suggested by Allen and Tildesley.[28] The solute–solvent interaction cut-off distance was based on all solute atoms.

Results and discussion

Structure and energetics from electronic structure calculations

We first discuss the minimum energy structures found for chorismate. We have identified three such structures (Fig. 1), which can be labelled as diaxial or diequatorial depending on the positions of the ether and hydroxy oxygen atoms with respect to the cyclohexadienyl ring. The diequatorial conformation (**5**) is found to be the most stable (Table 1) due to the intramolecular $OH\cdots O_2^-C$ hydrogen bond. Two diaxial conformers have been identified, the lower in energy of the two (**6**) has the enol pyruvoyl moiety positioned to avoid steric repulsion with the cyclohexadienyl ring. In the higher energy diaxial conformer (**7**), such steric interaction can be seen to be present. However, it is this latter diaxial conformer that has a similar structure to the transition state (**3**). We note that Wiest and Houk[15] were unable to locate this diaxial structure (**7**). The transition state (Fig. 2) involved in this reaction has little of the conformational freedom of the reactant. However, in both the diaxial conformers (**6** and **7**) and transition state (**3**) there are different possible orientations of the OH group, which we have investigated. For the transition state, a stationary structure with the OH group directed towards the ring (‘OHin’, **3a**) and one in which the OH group is directed away from the ring (‘OHout’, **3b**) were located, with structure **3a** being the more stable by *ca.* 3 kcal mol^{-1}. Stationary structures of the two reactant diaxial forms (**6** and **7**) could only be located having the OH group directed towards

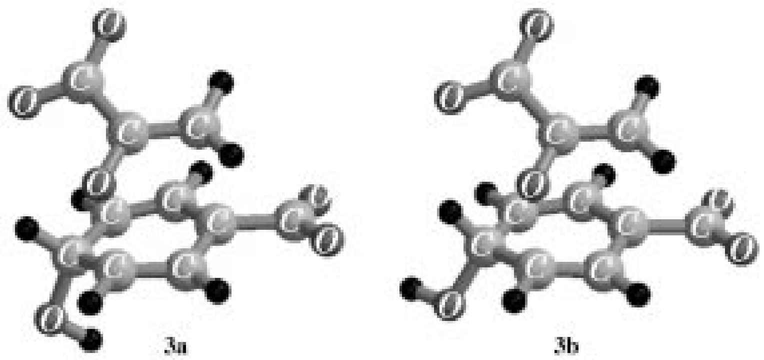


Fig. 2 Transition state structures of chorismate (**3**) with hydroxy group (OH) oriented ‘in’ (**3a**) and ‘out’ (**3b**)

Table 2 Relative free energies (kcal mol^{-1}) of chorismate, chorismic acid and transition state (TS) structures (298 K) from electronic structure calculations and Monte Carlo simulations

	Diequatorial	Diaxial		TS	
	5	**6**	**7**	OHin **3a**	OHout **3b**
Chorismic acid [6-31G*(HF)//6-31G*(HF)]	0	4.1	8.1	51.5	52.7
Chorismate [6-31G*(HF)//6-31G*(HF)]	0	10.9	17.0	65.7	68.9
Chorismate [6-31G*(MP2)//6-31G*(HF)]	0	10.8	16.0	35.7	40.1
Chorismate [6-31G*(PCM)//6-31G*(HF)]	0	3.8	9.4	50.7	51.6
Chorismate (Solvation energies, PCM)	0	−7.1	−7.6	−15.0	−17.3
Chorismate[a] (Monte Carlo simulation)	0	3.4 (3.3)	9.7 (8.7)		45.2 (16.4)
Chorismate (Solvation energies, Monte Carlo)	0	−7.5 ± 1.3	−7.3 ± 0.9		−23.7 ± 1.1 (−18.9 ± 0.5)[b]

[a] Values in parentheses employ the gas phase MP2 energies. [b] Using CHELPG charges from structure of ref. 15.

the ring. Corresponding structures with the OH group directed away from the ring were constructed and found to be higher in energy by a few kilocalories, in line with the results for the transition state.

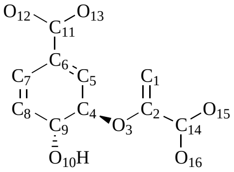
We draw the reader’s attention to the fact that some of our calculated energies (Table 1) differ slightly from those reported by Wiest and Houk.[15] In general this reflects the number of stationary structures of these flexible molecules, which are very close in energy. Thus, for the diequatorial conformer of chorismic acid, the difference in energy (0.8 kcal mol^{-1}) arises from the choice of different protonation sites of chorismate. For chorismate itself, our OHout (**3b**) structure for the transition state is 0.3 kcal mol^{-1} higher in energy. This is due to two possible orientations of the OHout group. The small energy difference between the two diaxial structures of chorismic acid is due to these structures being OHin and OHout in the present study and ref. 15, respectively. There is uncertainty as to the origin of the energy difference between the two diaxial chorismate structures, since we were unable to reproduce the quoted energy from the structure given in ref. 15.

In Table 2 we show the relative free energies of the various structures in the gas phase and in aqueous solution. Here the usual thermodynamic corrections have been obtained for the chorismate [6-31G*(HF)//6-31G*(HF)] structures, these values being assumed to be the same for the MP2 and PCM calculations. The calculated energy differences between the three reactant structures (Tables 1 and 2) clearly demonstrate both the strong intramolecular hydrogen bond and the different steric interactions in the two diaxial conformers. Similar effects are evident in the corresponding structures of chorismic acid, although the intramolecular hydrogen bond is, as expected, considerably weaker than in the dianion. For chorismate, the relative energies of these three conformers are little altered by the inclusion of electron correlation at the MP2 level. Correlation does, however, have a considerable effect on the calculated barrier height. Thus, with respect to the most stable structure in the gas phase (diequatorial), the barrier is 35.7 kcal mol^{-1} at MP2, compared to 65.7 kcal mol^{-1} at the HF level.

We turn now to the effect of solvation as modelled with the PCM approach (Table 2). For the reactant structures (**5**–**7**) we see that the solvation energies of the diaxial forms (**6** and **7**) are, as expected, considerably greater than those of the diequatorial form (**5**), due to the loss of the intramolecular hydrogen bond in the diaxial structures. However, this increased solvation energy is not sufficient to change the energy order found for the gas phase species, with the diequatorial form still being some 4 kcal mol^{-1} more stable than the diaxial form. The transition state structures (**3**) are considerably more strongly solvated than the reactant structures due to the enhanced polarity of these structures (Table 3), an effect seen in other Claisen rearrangements (*e.g.* that of allyl vinyl ether).[29–33] Of the two transition state structures (**3a** and **3b**), the one with the OH group directed away from the ring (**3b**) is more strongly solvated, by *ca.* 2 kcal mol^{-1}, than the one with OH group directed towards the ring (**3a**). This differential solvation reduces the energy difference between these forms in water to less than 1 kcal mol^{-1}. Thus, they are essentially isoenergetic and the PCM calculation cannot decide, with confidence, which form predominates in aqueous solution. A similar preferential solvation was given by the PCM approach for the reactant diaxial structures which we have constructed with the OH group directed away from the ring.

We now discuss the structure of the transition states and the related KIEs. The transition state involved in this reaction has little of the conformational freedom of the reactant, except for the orientation of the ring OH group, which we have already

Table 3 CHELPG charges of chorismate, reactant and transition state in the gas phase



	Diequatorial	Diaxial		TS	
Structure[a]	**5**	**6**	**7**	**3b**	**3b**[b]
C(H_2)-1	−0.237	−0.248	−0.238	−0.245	−0.228
C-2	0.335	0.303	0.308	0.419	0.384
O-3	−0.624	−0.628	−0.615	−0.684	−0.633
C(H)-4	0.563	0.674	0.615	0.632	0.399
C(H)-5	−0.296	−0.274	−0.236	−0.636	−0.560
C-6	−0.019	−0.046	−0.059	0.552	0.572
C(H)-7	−0.005	0.119	0.113	−0.219	−0.268
C(H)-8	−0.315	−0.453	−0.421	−0.075	0.012
C(H)-9	0.572	0.592	0.563	0.407	0.478
O(H)-10	−0.404	−0.435	−0.430	−0.447	−0.436
C-11	0.893	0.875	0.862	0.758	0.740
O-12	−0.838	−0.841	−0.825	−0.815	−0.800
O-13	−0.844	−0.829	−0.831	−0.802	−0.807
C-14	0.791	0.815	0.830	0.829	0.805
O-15	−0.770	−0.795	−0.805	−0.826	−0.823
O-16	−0.802	−0.829	−0.831	−0.848	−0.835

[a] Charges are united-atom CHELPG charges from 6-31G*(HF) structures. [b] Values from ref. 15.

Table 4 Bond lengths (Å) of chorismate, chorismic acid and transition states [6-31G*(HF)]

	Chorismic acid		Chorismate	
	C-4–O	C-1–C-6	C-4–O	C-1–C-6
Diequatorial **5**	1.425		1.422	
Diaxial **6**	1.422		1.419	
Diaxial **7**	1.426		1.416	
Transition state OHin **3a**	2.051	2.389	1.987	2.388
Transition state OHout **3b**	2.076	2.409	2.032	2.450

Table 5 Calculated tritium secondary kinetic isotope effect for the Claisen rearrangement of chorismate (298 K)[a]

Reactant	Transition state	C-4	C-1
5	**3a**	1.127	0.894
6	**3a**	1.255	0.873
7	**3a**	1.226	0.910
5	**3b**	1.141	0.913
6	**3b**	1.271	0.892
7	**3b**	1.242	0.930
		(1.149)[b]	(0.992)[b]

[a] Experimental values in parentheses. [b] Values from ref. 34.

discussed. The preference for this group to be oriented towards the ring is found in the transition states of both chorismic acid and chorismate, although the calculated energy difference between these two conformers is reduced from 3.2 kcal mol^{-1} in the anion, to 1.2 kcal mol^{-1} in the acid. The calculated bond lengths of the transition state are also dependent upon the OH group orientation (Table 4). In all cases the transition state is quite loose, with both the breaking (C-4–O) and forming bonds (C-1–C-6) being quite long. There are however, definite changes in the structure of the transition state related to the position of the OH group. The OHin structures lead to somewhat tighter transition states, with shorter C-4–O and C-1–C-6 bonds. This effect is especially evident for the anion. The possibility of different conformations of both the reactant and transition state naturally leads to a range of predicted KIEs. In Table 5 we summarise our calculated tritium secondary kinetic isotope effects for the anionic reaction. As far as the reactant structures are concerned, the major effect is the substantially smaller value at C-4 for the diequatorial structure (**5**). For the two transition state structures (OHin **3a**; OHout **3b**) the KIEs at both C-1 and C-4 are smaller for the more stable structure (OHin), having shorter C-4–O and C-1–C-6 bond lengths. An additional complication when assessing these predictions is that experimental data are only available for the aqueous reaction. Here the KIEs are 1.149 and 0.992 at C-4 and C-1, respectively.[34] It can be seen that for the KIE at C-4, the best agreement between theory and experiment is for the reactant in the diequatorial conformation (**5**) and the transition state having the OHout structure (**3b**).

It is of interest that Gajewski and Brichford have found for the related Claisen rearrangement of allyl vinyl ether,[35] that the deuterium KIE at C-4 is reduced in aqueous media, compared to the gas phase value and the value at C-1 is little changed. If such a trend is also true for chorismate, then the gas phase values of the KIE at C-4 that we predict for the diaxial and OHin conformations of the reactant and transition state, respectively, (1.23–1.26) are in line with the experimental value of 1.149 for the aqueous reaction. The corresponding values we predict at C-1 (0.87–0.91) are somewhat smaller than the experimental value. However, these arguments are clouded by the failure of theoretical studies to predict a reduced KIE at C-4 in aqueous media for the rearrangement of allyl vinyl ether.[29]

Monte Carlo simulations

The results of the FEP simulations are summarised in Table 2 along with the total free energy changes associated with the various transformations occurring in aqueous solution arrived at by combining the relative solvation free energies with the internal free energies of the various structures calculated at the [6-31G*(HF)//6-31G*(HF)] level. Thus, as was found from the continuum calculations, explicit solvation considerably reduces the energy separation of the different conformers from the gas phase values, but the diequatorial form is still predicted to be the most stable. To study the effect of explicit solvation on the orientation of the OH group, we have carried out FEP simulations to obtain the difference in the solvation free energies between the structures with the OH group directed towards and away from the ring, for both of the diaxial conformers (**6** and **7**). In these simulations we have taken the formal atomic charges to be independent of the orientation of the OH group, to avoid unrealistic solvation energy differences which arise when a different set of charges is used. A similar strategy is used by Jorgensen *et al.*[36] in studying solvation contributions to the anomeric effect in aqueous solution. We here use CHELPG atomic charges obtained for the OHout structures. For both conformers, solvation favours the structure with the OH group directed away from the ring, by 3.3 ± 1.1 and 3.1 ± 0.4 kcal mol^{-1} for (**6**) and (**7**), respectively. These values are somewhat larger than those given by the PCM calculations and again suggest that in solution, the OH group is quite flexible.

The strong solvation of the transition state leads to a reduction in the barrier to the reaction upon solvation of 23.7 kcal mol^{-1}. If we consider the barrier with respect to the diequatorial form (**5**), then the gas phase value including electron correlation [6-31G*(MP2)//6-31G*(HF)] of 40.1 kcal mol^{-1} is reduced to 16.4 kcal mol^{-1} in aqueous solution. However, we note that the use of the structures for the transition state (OHout, **3b**) given by Wiest and Houk[15] leads to somewhat different atomic charges than we have used (Table 3), in spite of the small energy difference between the structures (Table 1). The sensitivity of potential derived charges to molecular conformation is well known[37] and may be a serious hazard in simulation studies. To assess this effect, we have carried out FEP simulations to estimate the difference in solvation free energies between these two transition state structures, both being of OHout conformation. This lead to our structure having a greater solvation energy than that of Wiest and Houk by *ca.* 4.8 kcal mol^{-1} reflecting the greater charge separation given by our structure (Table 3). Thus, we note that differences in solvation energies estimated by such an approach utilising potential derived charges far outweigh gas phase conformational energy differences, thus making prediction of the solute conformation somewhat difficult.

Conclusions

Both the continuum approach and the Monte Carlo FEP model have inherent deficiencies. The continuum model suffers from the well-known lack of explicit solute–solvent intermolecular interactions. These are present in the Monte Carlo simulation, but their magnitude depends quite critically on conformationally dependant potential derived charges. However, a number of features of the structure and energetics of the various conformations of the reactant and transition state of the rearrangement of chorismate to prephenate have been identified, using both a continuum model and explicit solvation. It is worth noting that both approaches lead to very similar relative solvation energies for the structures we have studied (Table 2), giving added confidence in the reliability of the results obtained. A recent study[38] of a Menshutkin-type reaction in solution compared an *ab initio* continuum approach with a Monte Carlo simulation employing a hybrid QM/MM potential[39] and came to a similar conclusion, in spite of the problem of the treatment of the outlying charge in continuum solvation models which has been recently discussed.[40]

An important conclusion of our calculation is that the reactant is considerably more flexible in aqueous solution than in the gas phase. Thus, the energy separation between the diequatorial and diaxial conformers is considerably reduced, and the OH group is quite flexible in the diaxial structures. Copley and Knowles have studied the conformational equilibrium of chorismate in solution *via* NMR spectroscopy,[41] and have found that although the diequatorial form is dominant, 10–40% of the diaxial form is present in aqueous solution. Our MC simulations, combined with gas phase electronic structure calculations (Table 2) predict the diequatorial form (**5**) to be more stable than the diaxial structure (**6**), OHin, by *ca.* 3 kcal mol^{-1}. Thus, the experimental data suggest that we have somewhat overestimated the energy difference between the diaxial and diequatorial forms in aqueous solution. As far as the transition state structures are concerned, we predict significantly enhanced solvation energies compared to all of the reactant structures. The barrier to the reaction (from the diequatorial form) is predicted by the MC simulation, to be reduced by 23.7 kcal mol^{-1} due to the aqueous environment, a value considerably larger than that for the Claisen rearrangement of allyl vinyl ether (*ca.* 4 kcal mol^{-1}).[42,29] When used in conjunction with gas phase MP2 calculations, the barrier in solution is predicted to be 16 kcal mol^{-1}. In the case of the Claisen rearrangement of allyl vinyl ether, a calculation at the MP2 level[21] underestimates the gas phase barrier by 6 kcal mol^{-1}. Thus, we would expect a barrier for the reaction involving chorismate in aqueous solution to be in the region of 22 kcal mol^{-1}, a value very close to the experimental value[43] of 24.5 kcal mol^{-1}.

As far as the corresponding enzymic reaction is concerned, the increased flexibility of chorismate in aqueous solution allows the enzyme to recognise the diaxial conformer (**6**), followed by the appropriate conformational and structural changes accompanying the catalytic reaction. Our previous study of this reaction,[16] using a hybrid QM/MM approach has yielded a preferential binding of the transition state compared to the diaxial reactant, of 27 kcal mol^{-1}, compared to a corresponding value in water of 16 kcal mol^{-1} predicted from our Monte Carlo simulations, showing the strong catalytic effect of the enzyme active site.

It is worth commenting on the use of gas phase Hartree–Fock charges used in the MC simulations. Such an approach may be criticised for the neglect of both solute polarisation and electron correlation effects. The former will lead to increased charge separation whilst correlation usually results in a reduction in molecular polarity. Thus, it may be argued that these two effects partially cancel out leading to formal atomic charges close to the gas phase Hartree–Fock values. This has been confirmed by detailed calculations on the Claisen rearrangement of allyl vinyl ether[29] and provides an explanation of the widespread success of such MC simulations using gas phase Hartree–Fock atomic charges.

As in the case of allyl vinyl ether, the structure of the transition state corresponds to considerable bond weakening and little bond formation, in line with the measured and predicted KIEs at C-4 and C-1. Comparison between the calculated and measured KIEs suggests there is less bond formation than predicted by our gas phase Hartree–Fock calculations. We note that for allyl vinyl ether, both the inclusion of electron correlation *via* density functional methods[44] and of solvation[45] result in reduced bond formation, so that our underestimation of the KIE at C-1 is not unexpected.

Whilst this work was being prepared for publication, a Monte Carlo FEP simulation of chorismate solvation, using the structures and charges of Wiest and Houk,[15] appeared in the press.[46] Whilst there is some overlap with the present work, our study addresses further issues of structural flexibility as well as describing the results of continuum calculations and discussion of the kinetic isotope effects.

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