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Review Commentary

Flash photolytic generation and investigation of short-lived reaction intermediates: a case study

A. J. Kresge*

Department of Chemistry, University of Toronto, Toronto, Ontario M5S 3H6, Canada

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ABSTRACT: The advantage of adding more structure-diagnostic information to the simple detection of flash photolytically generated transient species by changes in UV–visible light absorbance is illustrated by a case study involving the mandelic acid keto–enol system. An early report based on preliminary evidence proposed that flash photolysis of phenyldiazoacetic acid produces the enol of mandelic acid by hydration of phenylhydroxyketene, itself generated by a photo-Wolff reaction of the diazo acid. Further examination, however, shows that this is only a minor route, and that the major pathway is a new enol-forming reaction involving what appears to be hydration of a carboxycarbene formed by dediazotization of the diazo compound. Hydration of phenylhydroxyketene is nevertheless the reaction by which mandelic acid enol is generated when esters of benzoylformic acid are the flash photolysis substrates. These mechanisms, and also identification of the enol as a tranisent species, are supported by detailed arguments involving acid–base catalysis, solvent isotope effects, and the use of oxygen-18 as a tracer. The work produces a keto–enol equilibrium constant for the mandelic acid system, $pK_E = 16.19$, and also acidity constants of the enol ionizing as an oxygen acid, $pK^E_a = 6.39$, and the keto isomer ionizing as a carbon acid, $pK^K_a = 22.57$. The bearing of these results on the enzyme-catalyzed racemization of mandelic acid is discussed. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: Flash-photolysis; short-lived reaction intermediates; mandelic acid; keto–enol tautomers; enzyme-catalyzed racemization

INTRODUCTION

In the 10 years since the *Journal of Physical Organic Chemistry* was founded, flash photolysis has developed from being a technique used principally by photochemists to gain information about excited states to being a tool commonly employed by physical organic chemists to investigate the chemistry of short-lived reaction intermediates. It has proved to be especially effective in providing a wealth of new information about carbocations and enols.[1] Of the fast reaction techniques available, flash photolysis is probably the best suited for this new purpose. Relaxation methods such as temperature jump involve perturbing an equilibrium that is not too far to one side or the other, and they are consequently not suited for studies of very unstable species that exist only far from equilibrium, and stopped-flow methods are limited to the millisecond time range.

Flash photolysis, of course, is also not without its limitations. It requires a photochemical reaction to generate the substance to be investigated. It also suffers from the fact that the two properties most commonly used to monitor the concentrations of transient species in flash photolysis work, UV–visible light absorbance and solution conductivity, are not especially structure diagnostic, and they consequently serve only poorly to identify the short-lived substances observed. The latter limitation can be overcome in part by using infrared absorbance to interrogate the reacting solution,[2] but this method of detection is not suited for studies in aqueous solution because water itself absorbs infrared light strongly.

In our investigations of short-lived reaction intermediates by flash photolysis, we have compensated for this structure-diagnostic limitation of UV–visible light detection by performing chemistry on the transient species observed; for example, by determining how their lifetimes are affected by acid or base catalysts, or by isotopic substitution, and then using the mechanistic information so gained in much the same way as physical organic chemists commonly do to investigate slow reactions. We illustrate this in the present review by describing a recently completed study of the enol of mandelic acid.[3] Under the conditions of this investigation (25 °C, aqueous solution), the position of equilibrium between the very

**Correspondence to:* A. J. Kresge, Department of Chemistry, University of Toronto, Toronto, Ontario M5S 3H6, Canada
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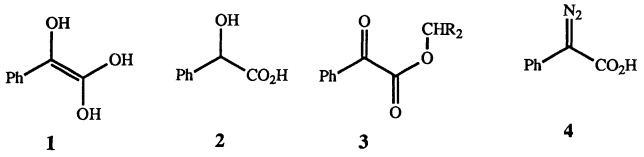
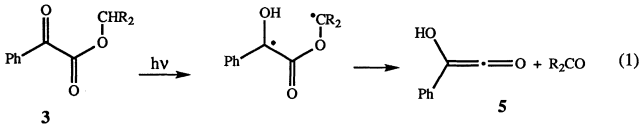
labile enol and its much more stable keto isomer was 16–19 orders of magnitude to one side; examination of this system by relaxation methods was therefore impossible. Most of the rates of reaction we encountered, moreover, were beyond the upper limit of the stopped-flow time range. This case study also shows how, by gathering more mechanistic information, we were able to rectify a preliminary misidentification of one of the enol precursors.[4]

MANDELIC ACID ENOL

We generated the enol, **1,** of mandelic acid, **2,** in two different ways: by flash photolysis of esters of benzoylformic acid, **3,** and by flash photolysis of phenyldiazoacetic acid, **4.** With

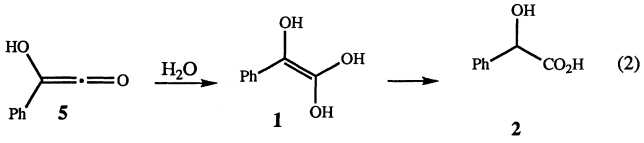


both kinds of substrate, we observed a rapid rise and then a somewhat slower decay of absorbance in the region, $\lambda \approx 300$ nm, where we expected the styrene-type chromophore of mandelic acid enol might absorb. That, plus the fact that previous work[5] had shown that flash photolysis of benzoylformates such as **3** leads to Norrish type-II photoelimination producing phenylhydroxyketene, **5,** [equation (1)] which, in our aqueous medium, would be hydrated rapidly by



(1)

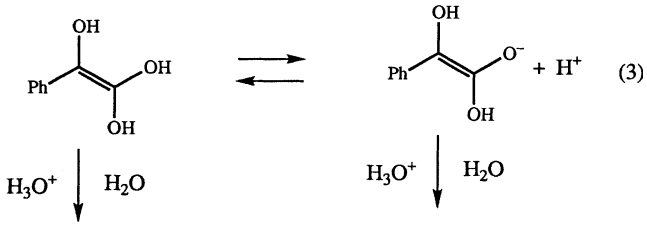
nucleophilic addition of water to its carbonyl group,[6] suggested that the absorbance changes we were observing were caused by the formation and subsequent ketonization of mandelic acid enol [equation (2)].



(2)

Although mandelic acid is the principal product formed by flash photolysis of benzoylformate esters and phenyldiazoacetic acid in aqueous solution, other substances are also produced in minor amounts. The possibility did exist, therefore, that we were observing, not the formation and ketonization of mandelic acid enol, but one of these minor reactions instead, or perhaps even the formation and decay of some other intermediate in the mandelic-acid-generating pathway. More evidence was needed to settle this issue.

The ketonization of enols is known to occur by rate-determining protonation of the β-carbon atom of either the enol or the enolate ion, according to the reaction scheme of equation (3).[7]



(3)

This mechanism produces a characteristic rate profile for reaction through solvent-derived species; it also requires the reaction to show general acid catalysis and have hydronium ion isotope effects in the normal direction ($k_H/k_D > 1$).

The rate profile for decay of the transient species that we observed, shown in Fig. 1 provides good evidence for the reaction scheme in equation (3). This profile contains an acid-catalyzed portion at low $[H^+]$, as expected for carbon protonation of non-ionized enol by the hydronium ion. That is then followed by a short 'uncatalyzed' region at $[H^+] = 10^{-2}$–10^{-3} M, which represents either protonation of enol by a water molecule, or ionization of the enol to the much more reactive enolate ion followed by carbon protonation of enolate by hydronium ion; in the latter case, a hydronium ion is produced in a rapid pre-equilibrium and is then used up in the rate-determining step, which gives the overall process the appearance of an 'uncatalyzed' reaction. This portion of the rate profile is followed by a region of apparent hydroxide ion catalysis in which ionization of enol to enolate is followed by carbon protonation of enolate by a water molecule; a hydronium ion is produced in the pre-equilibrium but is then not used up, making the rate of the overall process inversely proportional to $[H^+]$ and giving it the appearance of a hydroxide ion-catalyzed process. Finally, when the position of the enol–enolate pre-equilibrium shifts over to enolate ion, this hydroxide ion catalysis becomes saturated and another 'uncatalyzed' portion of the rate profile, corresponding to simple rate-determining carbon protonation of enolate by water, results.

In buffer solutions of low $[H^+]$, where hydroxide ion catalysis is saturated, decay of our transient showed general acid catalysis, as expected for rate-determining carbon protonation of the enolate ion. In more acidic buffers, however, in the region where hydroxide ion catalysis is operative, the reaction showed general base catalysis. This, too, is as expected for the reaction scheme in equation (3), because, although enolate ion is the reactive form of the substrate in these solutions, non-ionized enol is the dominant form; conversion of enol into enolate then introduces an inverse rate dependence on $[H^+]$, which converts the general acid catalysis of the rate-determining carbon protonation step into an observed general base catalysis.

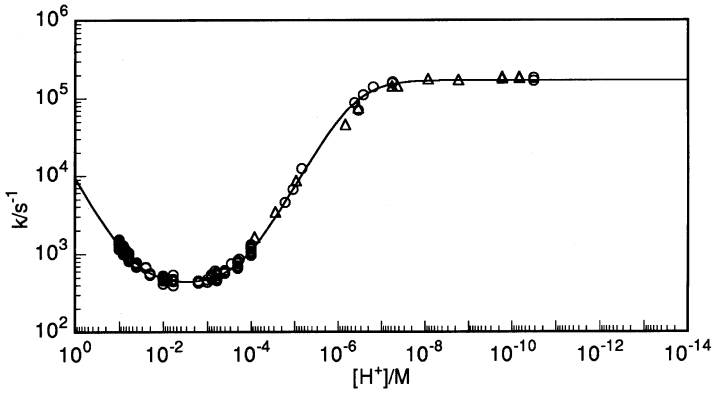


Figure 1. Rate profile for decay of the transient species identified as the enol of mandelic acid, generated by flash photolysis of (o) benzoylformic acid esters and (Δ) phenyldiazoacetic acid

General acid catalyzed reactions are expected to conform to the Brønsted relationship, and the catalytic coefficients for decay of our transient do give a good correlation. This correlation, moreover, has a low Brønsted exponent, $\alpha = 0.24$, which implies an early, reactant-like transition state for the process it represents;[8] this again is consistent with the expected strongly exoergic nature of the mandelic acid enolate ion ketonization reaction.[9]

Solvent isotope effects provide additional support for the interpretation of our transient decay as an enol ketonization reaction. Comparison of rates of reaction in H_2O and D_2O in the region of acid catalysis at high acidity gave $k_H/k_D = 3.2$, and a similar comparison in the region of saturation of hydroxide ion catalysis gave $k_H/k_D = 6.9$. Both of these isotope effects are in the normal direction ($k_H/k_D > 1$), as expected for rate-determining hydron transfer to carbon. The relative magnitude of these isotope effects, moreover, is also as expected, for the scheme in equation (3) would assign the first and smaller one to hydron transfer from the hydronium ion, where a primary isotope effect in the normal direction is offset by an inverse ($k_H/k_D < 1$) secondary component, and it would assign the second and larger effect to hydron transfer from a water molecule, where a normal primary isotope effect is reinforced by a secondary component in the normal direction.[10]

Interpretation of the rate profile in Fig. 1 in terms of the reaction scheme in equation (3) allows of the break in the profile at $[H^+] \approx 10^{-6}$ M to be assigned to acid ionization of the enol, and our comparison of rates of reaction in H_2O and D_2O provides an isotope effect on the equilibrium constant for this ionization, $K_H/K_D = 4.5$. This, again, is consistent with expectation, for isotope effect theory requires oxygen acids to be less completely ionized in D_2O than in H_2O,[10] and the magnitude of the effect observed here is reasonable for an acid of the strength of this enol ($pK_a = 6.39$).[11]

A variety of standard mechanistic criteria thus paint an overall consistent picture and provide strong support for identification of the transient species we have observed as the enol of mandelic acid.

ENOL PRECURSORS

Identification of our transient as the enol of mandelic acid, coupled with the facts that phenylhydroxyketene is

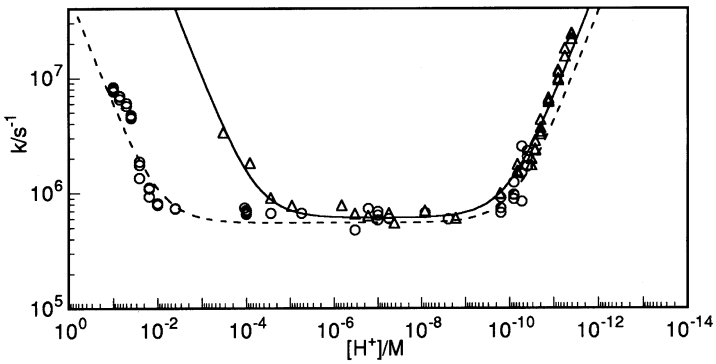


Figure 2. Rate profiles for formation of mandelic acid enol from (o) benzoylformic acid esters and (Δ) phenyldiazoacetic acid

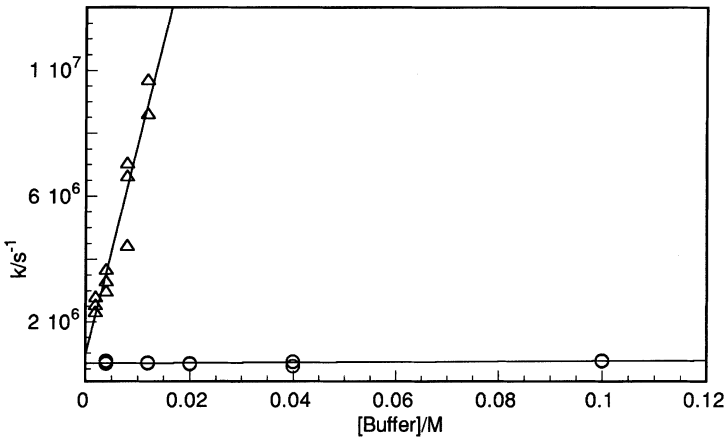
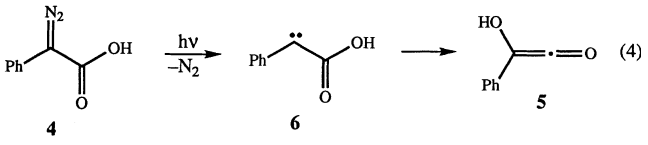


Figure 3. Buffer dilution plots for the formation of mandelic acid enol from (o) *n*-butyl benzoylformate and (Δ) phenyldiazoacetic acid (acetic acid buffers, buffer ratio = 1, ionic strength = 0.10 M)

formed by flash photolysis of benzoylformic acid esters[5] and ketenes are known to hydrate rapidly to carboxylic acid enols,[6] makes a strong case for designating hydration of this ketene as the enol-forming reaction when benzoylformic acid esters are used as the flash photolysis substrates [equations (1) and (2)]. This assignment is supported by the rate profile for enol formation defined by the circles shown in Fig. 2. Ketene hydration rate profiles characteristically have long uncatalyzed portions, with weak hydroxide ion catalysis and also weak or non-existent hydronium ion catalysis,[12] just like the rate profile shown in Fig. 2. Solvent isotope effects on the uncatalyzed reaction, moreover, are generally weak,[12b,d,13] as is the isotope effect found here, $k_H/k_D = 1.49$, consistent with the fact that this reaction involves nucleophilic attack of water on the ketene carbonyl carbon atom,[6] with some weakening but no breaking of isotopically substituted bonds.

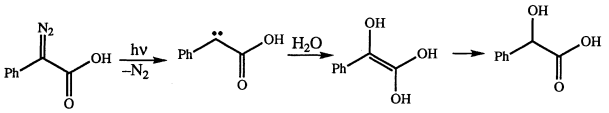
Our early, rather limited studies with the other flash photolysis substrate, phenyldiazoacetic acid, **4,** were confined to neutral and basic solutions where, as the triangles in Fig. 2 demonstrate, rates of this reaction are indistinguishable from those obtained using benzoylformate esters as the substrate (Fig. 2 circles). Since we had established that phenylhydroxyketene was the enol precursor in the benzoylformate ester reaction, we assumed that this ketene was also the precursor in the phenyldiazoacetic acid reaction. We postulated that the ketene was formed in this case by a Wolff rearrangement of the carboxycarbene, **6,** generated by photo-induced loss of nitrogen from the diazo compound, [equation (4)], and we reported that in a preliminary publication of part of this work.[4]



(4)

We discovered later, however, that, as Fig. 2 shows, rates of formation of mandelic acid enol from the two kinds of flash photolysis substrate are decidedly different in acidic solutions. The behavior of the two systems is also different in buffer solutions: as Fig. 3 demonstrates, whereas formation of the enol using phenyldiazoacetic acid as the substrate is catalyzed strongly by acetic acid buffers, that using a benzoylformate ester is not. Similar differences were seen in all of the buffers we examined up to $[H^+] = 10^{-9}$ M. However, both reactions produce the same enol, as evidenced by the fact that the circles and triangles in Fig. 1, which represent data obtained with benzoylformate esters and phenyldiazoacetic acid, respectively, make up a single rate profie. It is clear, nevertheless, that the two different kinds of substrate generate the enol by different reactions, through different enol precursors, in both acidic and basic solutions.

Photolysis of diazo compounds is known to produce carbenes,[14] and insertion of carbenes into O—H bonds is a well known reaction.[15] It seems likely, therefore, that this is the process that occurs upon flash photolysis of phenyldiazoacetic acid in aqueous solution. Our observation of an enol intermediate, however, shows that the insertion is not a direct reaction of the carbenic carbon atom only, but that it also involves the carbonyl group of the carboxylic acid function, and is more in the nature of a conjugate addition of water across the entire carbonylcarbene moiety [equation (5)]. We have observed similar enol-forming photo-dediazotizations of the methyl ester

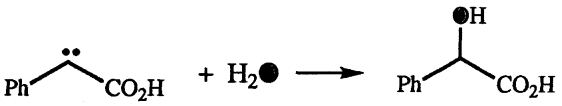


(5)

of phenyldiazoacetic acid[16] and of its cyclic analog, 4-diazo-3-isochromanone.[17] We are currently investigating the mechanism of these reactions. It is noteworthy that the lifetimes of our postulated carbonylcarbene inter-

mediates are considerably longer than those of other carbonylcarbenes without phenyl substituents that have been observed recently in non-aqueous solvents.[18]

Further insight into the nature of the enol-forming process when phenyldiazoacetic acid is used as the flash photolysis substrate comes from an oxygen-18 tracer study that we conducted in isotopically labelled water. The carbonylcarbene route will give mandelic acid with the isotopic label in its α-hydroxyl group [equation (6)], whereas hydration of phenylhydroxyketene will give mandelic acid with the isotopic label in its carboxyl group [equation (7)]. These two oxygen positions



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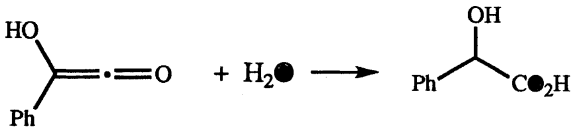
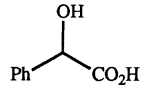
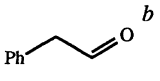


(7)

can be distinguished easily by mass spectrometry, because mandelic acid fragments readily upon electron impact, producing the phenylhydroxymethyl cation as the principal species in its mass spectrum.[19] Our results showed that 94% of the mandelic acid formed from phenyldiazoacetic acid in acidic solution was labelled in its α-hydroxyl group and 4% was unlabelled at that position, thus supporting the carbonylcarbene route as the dominant enol-generating reaction in that medium, but suggesting that hydration of phenylhydroxyketene formed by the photo-Wolff reaction in equation (4) might in fact be making a minor contribution. The occurrence of such a minor route is supported by the fact that the amount of α-hydroxyl group labelled mandelic acid dropped to 80% when the experiment was performed in basic solution. In basic solution the carboxylic acid group of the carbene intermediate will be ionized, and O^- rather than OH can be expected to be the migrating moiety in the Wolff rearrangement. Since this rearrangement is to an electron deficient center, the migrating aptitude of O^- should be greater than that of OH, and in basic solution the Wolff rearrangement will be able to compete more effectively with carbonylcarbene hydration. The latter reaction, however, appears still to be the dominant enol-generating process when phenyldiazoacetic acid is the flash photolysis substrate, even in basic solution.

Our detailed investigation of enol precursors has thus revealed a complexity, and has discovered a new enol-producing reaction, that was not at all apparent from our initial cursory examination of these systems.[4] This serves to illustrate the advantage of using standard mechanistic criteria to supplement simple UV–visible light detection of flash-photolytically generated transient species.

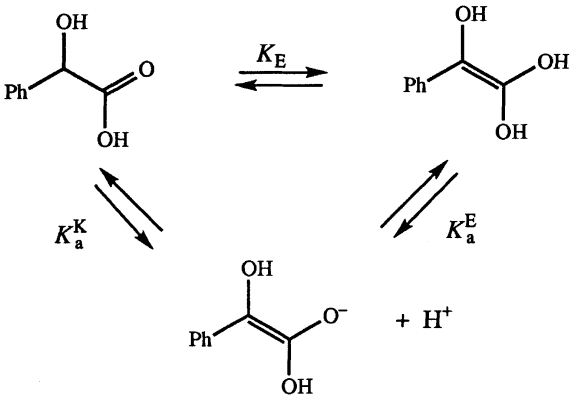
Table 1. Comparison of mandelic acid and phenylacetaldehyde keto–enol systems[a]

Constant		 [b]
pK_E^E	16.19	2.88[c]
pK_a^E	6.39	9.46,[d] 9.76[e]
pK_a^K	22.57	12.43[c]

[a]Aqueous solution, 25 °C.
[b]Ref. 20.
[c]Global constant referring *to cis* and *trans* isomers.
[d]*trans*-Enol.
[e]*cis*-Enol.

KETO–ENOL AND RELATED EQUILIBRIA

Keto–enol equilibrium constants, K_E, of simple carboxylic acids are too small to be measured by direct methods such as halogen titration, but they can be determined as ratios of enolization, k_E, to ketonization, k_K, rate constants: $K_E = k_E/k_K$. Combination of rates of ketonization of mandelic acid enol, measured flash photolytically, with much slower rates of enolization of the acid, measured as α-hydrogen exchange, then leads to the result $K_E = 6.48 \times 10^{-17}$, $pK_E = 16.19$.[3] As the thermodynamic cycle of equation (8) shows, this equilibrium constant may be combined with the acidity constant of the enol, $K_a^E = 4.12 \times 10^{-7}$ M, $pK_a^E = 6.39$, obtained from the ketonization rate profile, to determine the acidity constant of mandelic acid ionizing as a carbon acid, $K_a^K = 2.67 \times 10^{-23}$ M, $pK_a^K = 22.57$.



(8)

These results are compared with the corresponding constants for the phenylacetaldehyde system in Table 1. It can be seen that the keto–enol equilibrium constant for mandelic acid is many orders of mangnitude smaller than that for phenylacetaldehyde, a difference that may be attributed to resonance interaction between the carbonyl and hydroxyl moieties of the carboxyl group of mandelic acid, which stabilizes the keto isomer in this case and raises the energy difference between it and the enol. The acidity constant of mandelic acid enol, on the other hand, is greater than that of phenylacetaldehyde enol. The

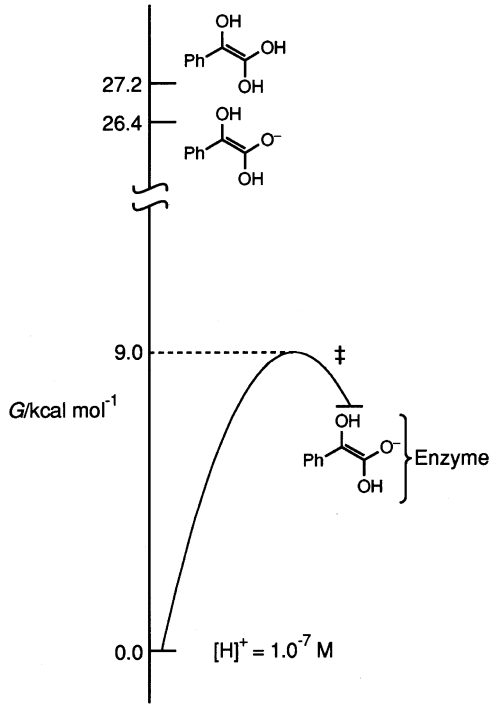


Figure 4. Energetic relationships for the mandelic acid keto–enol system in neutral solution

difference here, 3.1–3.4 pK units, is not unlike the 2.3 average pK_a difference between the acidity constants of a group of alcohols and the corresponding *gem*-diols,[21] which can be assigned to an inductive, anion-stabilizing effect of the second hydroxyl group; this effect might be transmitted more effectively through the sp^2 carbon atom of a carboxylic acid enol than through the sp^3 carbon atom of a saturated *gem*-diol, thus accounting for the greater pK_a difference in the case of the enols. The acidity constant of mandelic acid ionizing as a carbon acid, just like its keto–enol equilibrium constant, is many orders of magnitude greater than that of phenylacetaldehyde, and this again may be attributed to resonance stabilization of the non-ionized keto isomer.

MANDELATE RACEMASE

Mandelate racemase is a much-studied enzyme that catalyzes the racemization of mandelic acid very effectively. An impressive body of evidence shows that it does this by an enolization pathway,[22] which is a very slow process in the absence of the enzyme: in order to obtain measurable rates of enolization for our determination of K_E, we had to use concentrated acid solutions (0.5 – 4 M hydrochloric acid) and high temperatures (130–150 °C). The enzyme thus has a large kinetic barrier to overcome in catalyzing the racemization reaction. Our results show that it also has a sizable thermodynamic barrier.

This is illustrated by the free energy diagram in Fig. 4. This diagram shows the relative free energies of the substances involved, referred to a standard state of $[H^+] = 10^{-7}$ M that corresponds to the neutral solution in which the enzymatic reaction takes place. Interestingly, at this acidity mandelic acid enol and enolate ion have closely similar free energies and differ little in stability; either one of these species could consequently be involved in the enzymatic reaction with equal facility. Taking mandelate ion and free enzyme as the initial state of the enzymatic reaction and using $k_{cat} = 1070\ s^{-1}$ and $K_m = 0.63$ mM[23] give a rate constant that corresponds to the free energy of activation $\Delta G^{\ddagger} = 9.0$ kcal mol^{-1}. The transition state of the enyzmatic reaction thus lies some 17–18 kcal mol^{-1} below the free energy of mandelic acid enol or enolate ion. The enyzme must therefore stabilize its enolic intermediate by at least this amount if this intermediate lies at the same free energy level as the transition state, or by a somewhat greater amount, if, as seems likely, the intermediate lies below the transition state.

Two different hypotheses have been advanced to account for this stabilization: one attributes it to the formation of a strong 'low-barrier' hydrogen bond between the enolic intermediate and an essential glutamic acid residue at the active site of the enzyme,[24] and the other to electrostatic stabilization of the intermediate by the divalent metal ion cofactor essential for the enzymatic reaction.[25] Current opinion seems to favor the electrostatic explanation.[26]

Acknowledgments

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