The Mandelic Acid Keto−Enol System in Aqueous Solution. Generation of the Enol by Hydration of Phenylhydroxyketene and Phenylcarboxycarbene

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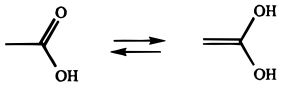
Abstract: Flash photolysis of phenyldiazoacetic acid and the methyl, *n*-butyl, and isobutyl esters of benzoylformic acid in aqueous solution generated a transient species that was identified, through product determination and the form of acid−base catalysis plus solvent isotope effects on its decay, as the enol of mandelic acid, **1**. When benzoylformate esters were the flash photolysis substrates, the enol was formed by hydration of phenylhydroxyketene, **5**, itself generated by Norrish type II photoelimination of the esters, and when phenyldiazoacetic acid was the substrate, the enol was formed by hydration of phenylcarboxycarbene, **6**, produced by dediazotization of the diazo acid. Rates of enolization of mandelic acid were also determined, by monitoring the incorporation of deuterium into its α-position from a D_2O solvent, and combination of these with rates of ketonization gave the keto−enol equilibrium constant, $pK_E = 16.19$. The acidity constant of the enol ionizing as an oxygen acid was determined as well, $pQ_a^E = 6.39$, and combination of that with K_E led to the ionization constant of the keto form of mandelic acid ionizing as a carbon acid, $pQ_a^K = 22.57$. (These acidity constants are *concentration quotients*, applicable at ionic strength = 0.10 M.) These results are compared with other keto−enol systems, and their bearing on the enzymatic racemization of mandelic acid is discussed.

Although the enol isomers of most simple aldehydes and ketones are quite unstable, both thermodynamically and kinetically, there has recently been a remarkable increase in our knowledge of their chemistry, enabled largely by the development of methods for generating these substances in solution in greater than equilibrium amount under conditions where they can be observed directly and their reactions can be studied in detail.[1] Application of these techniques to the enol isomers of simple carboxylic acids, however, is much more difficult, because carboxylic acid enols are very much more unstable. For example, the equilibrium constant for keto−enol isomerism of acetic acid in aqueous solution, eq 1, though not yet determined experimentally, has been reliably estimated at $pK_E = 20$;[2] this is 14 orders of magnitude less than the equilibrium



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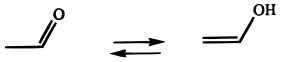
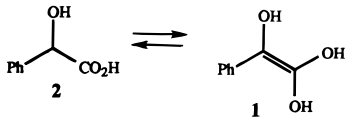
constant for the keto−enol transformation of acetaldehyde, eq 2, for which $pK_E = 6.23$.[3] A rate constant for the hydrogen-ion catalyzed ketonization of acetic acid enol may also be



(2)

estimated, using $k_H^+ = 7 \times 10^5\ M^{-1}\ s^{-1}$ for the hydrolysis of ketene dimethyl acetal,[4] which is the dimethyl ether of acetic acid enol, $CH_2{=}C(OMe)_2$, and the fact that methyl enol ethers undergo hydrogen-ion catalyzed hydrolysis one to two orders of magnitude more slowly than ketonization of the corresponding enols.[5] The result, $k_H^+ = 10^8-10^9\ M^{-1}\ s^{-1}$, is 7−8 orders of magnitude greater than $k_H^+ = 33\ M^{-1}\ s^{-1}$ for the ketonization of acetaldehyde enol.[3]

Daunting realities such as this have directed the so far quite limited studies of carboxylic acid enols to systems where the enol is stabilized, either sterically[6] or electronically.[7] Carbon−carbon double-bond-stabilizing substituents such as phenyl and hydroxyl[8] can be expected to be effective in this respect, and we have found that the enol, **1**, of mandelic acid, **2**, which contains both of these groups, though still quite reactive, is



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amenable to study.

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(3) Chiang, Y.; Hojatti, M.; Keeffe, J. R.; Kresge, A. J.; Schepp, N. P.; Wirz, J. *J. Am. Chem. Soc*. **1987**, *109*, 4000−4009.

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(7) (a) Urwyler, B.; Wirz, J. *Angew. Chem., Int. Ed. Engl*. **1990**, *29*, 790−792. (b) Chiang, Y.; Kresge, A. J.; Pruszynski, P.; Schepp, N. P.; Wirz, J. *Angew. Chem., Int. Ed. Engl*. **1990**, *29*, 792−794. (c) Barra, M.; Fisher, T. A.; Cernigliaro, G. J.; Sinta, R.; Scaiano, J. C. *J. Am. Chem. Soc.* **1992**, *114*, 2630−2634. (d) Andraos, J.; Chiang, Y.; Huang, C-G.; Kresge, A. J.; Scaiano, J. C. *J. Am. Chem. Soc*. **1993**, *115*, 10605−10610. (e) Andraos, J.; Chiang, Y.; Kresge, A. J.; Pojarlieff, I. G.; Schepp, N. P.; Wirz, J. *J. Am. Chem. Soc*. **1994**,*116*, 73−81. (f) Almstead, J.-I. K.; Urwyler, B.; Wirz, J. *J. Am. Chem. Soc*. **1994**, *116*, 954−956. (g) Andraos, J.; Kresge, A. J.; Popik, V. V. *J. Am. Chem. Soc*. **1994**, *116*, 961−967. (h) Andraos, J.; Chiang, Y.; Kresge, A. J.; Popik, V. V. *J. Am. Chem. Soc*. In press.

(8) (a) Hine, J. *Structural Effects on Equilibria in Organic Chemistry*; Wiley-Interscience: New York, 1975; p 273. (b) Keeffe, J. R.; Kresge, A. J. *J. Phys. Org. Chem*. **1992**, *5*, 575−580.

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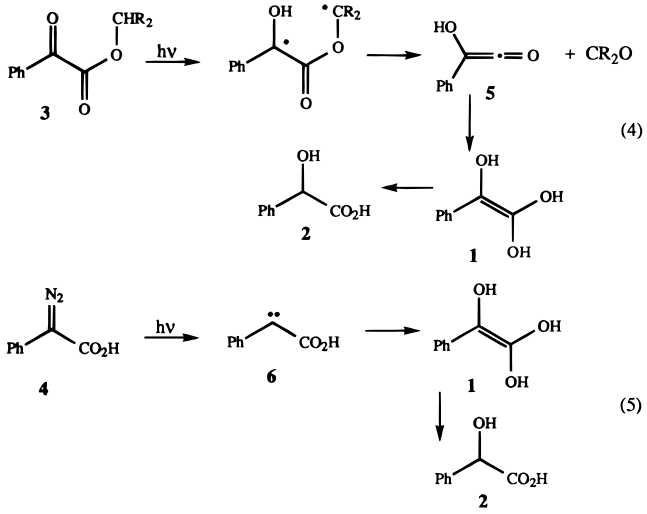
We generated the enol of mandelic acid flash photolytically using two different photochemical reactions: (1) Norrish type II cleavage of benzoylformic acid esters, **3**, eq 4, and elimination of nitrogen from phenyldiazoacetic acid, **4**, eq 5. The first of these reactions had been studied before in nonaqueous solvents



where phenylhydroxyketene, **5**, and substances derived from it were found to be produced;[9] in aqueous solution this ketene would be expected to be hydrated to mandelic acid enol, as shown in eq 4. In a preliminary publication of some of the presently described work,[7b] we postulated that photolysis of phenyldiazoacetic acid, **4**, also produced phenylhydroxyketene by a photo-Wolff reaction. We now know that this photo-Wolff reaction is only a minor route to mandelic enol from this substrate, and we believe instead that mandelic acid enol is generated from this substance principally by hydration of phenylcarboxycarbene, **6**, as shown in eq 5. These reaction paths are supported, as will be described below, by the form of acid−base catalysis and solvent isotope effects shown by transient absorbance changes produced by the flash photolysis of benzoylformic acid esters, **3**, and phenyldiazoacetic acid, **4**, as well as by isotopic tracer studies.

We have also measured the very slow rates of enolization of mandelic acid in order to evaluate the keto−enol equilibrium constant for this system, K_E, by combining the enolization rate constant, k_E, with the flash-photolytically determined ketonization rate constant, k_K, according to the relationship $K_E = k_E/k_K$. We orginally[7b] believed that acid-catalyzed racemization of mandelic acid occurred by an enolization pathway, but we now know that this is not so, and we have consequently used hydrogen exchange to monitor enolization.

The results that we have obtained constitute one of the first sets of detailed information on the chemistry of a simple, nonsterically hindered carboxylic acid enol. They also bear upon a much studied enzymatic reaction catalyzed by mandelate racemase[10] by providing a quantitative measure of the thermodynamic barrier that this enzyme must overcome; two different hypotheses have recently been advanced concerning the mechanism that the enzyme uses to overcome this barrier.[11]

(9) Encinas, M. V.; Lissi, E. A.; Zanocco, A.; Stewart, L. C.; Scaiano, J. C. *Can. J. Chem.* **1984**, *62*, 386−391, and references to earlier work cited therein.

(10) For a recent review see Kenyon, G. L.; Gerlt, J. A.; Petsko, G. A.; Kozarich, J. W. *Acc. Chem. Res.* **1995**, *28*, 178−186.

(11) (a) Gerlt, J. A.; Gassman, P. G. *J. Am. Chem. Soc.* **1993**, *115*, 11552−11568. (b) Guthrie, J. P.; Kluger, R. *J. Am. Chem. Soc.* **1993**, *115*, 11569−11572.

Experimental Section

Materials. *n*-Butyl and isobutyl benzoylformate were prepared by treating benzoylformyl chloride with the corresponding alcohol,[9] and phenyldiazoacetic acid was prepared by saponification of methyl phenyldiazoacetate, itself obtained by lead tetraacetate oxidation of the hydrazone of methyl benzoylformate.[12] The diazoacid undergoes rapid acid-catalyzed hydrolysis,[13] and it was therefore not isolated as such. Aqueous stock solutions of the acid salt were used instead; these were obtained by hydrolysis of methyl phenyldiazoacetate with an equimolar amount of 0.1 M aqueous sodium hydroxide and then washing the resulting solution with methylene chloride to remove any unreacted ester.

Acetylmandelic acid was prepared by treating mandelic acid with acetyl chloride.[14]

All other materials were best available commercial grades.

Kinetics. Flash photolysis was performed using a conventional flash lamp system and an excimer-laser system that have already been described.[3,7d] The conventional system produced a 50-μs excitation pulse and the laser system, operating at $\lambda = 248$ nm, produced a 20-ns excitation pulse. In both systems the temperature of the reacting solutions was controlled at 25.0 ± 0.05 °C.

Rates of hydrogen exchange were measured using ^{1}H NMR to monitor incorporation of deuterium from a D_2O solvent into the α-position of mandelic acid. The area of the α-proton signal was determined by comparison with that of the aromatic protons; further comparison of the latter with the signal from tetramethylammonium chloride, used as an internal frequency standard, showed that the aromatic hydrogens did not exchange under the rather drastic experimental conditions needed to effect α-hydrogen exchange. Rate measurements were made in concentrated DCl solutions (0.5−4 M) over the temperature range 130−150 °C. Corrections for changes in acid concentration between the temperature at which the solutions were prepared (25 °C) and those at which the reactions were run were made using polynomial relationships between density and temperature.[15] These relationships covered H_2O up to 150 °C, but they covered D_2O only up to 100 °C; they showed, however, that the ratio of the density of H_2O to that of D_2O remains virtually constant above 50 °C, and the temperature coefficient of H_2O density was therefore used to calculate D_2O densities at 130−150 °C, on the assumption that this constancy extended to this range. All exchange reactions were monitored for several half-lives. The data obtained fit the first-order rate law well, and observed first-order rate constants were calculated by least-squares fitting of an exponential function.

Rates of racemization were measured using (*R*)-(−)-mandelic acid as the substrate. High temperatures and concentrated acids, comparable to those used for hydrogen exchange measurements, were again required to effect reaction. These data also fit the first-order rate law well, and observed first-order rate constants were once again obtained by least squares fitting of an exponential function.

Results

Product Studies. Product studies were carried out by subjecting wholly aqueous solutions of substrate, at concentrations comparable to those used for the flash photolytic rate measurements (*ca.* 10^{-5} M), to a single flash from our conventional flash photolysis system and then analyzing the resulting solutions by HPLC. Identities of substances appearing in the chromatograms were established by comparing retention times and UV spectra and also by spiking with authentic samples. Analyses were performed for methyl benzoylformate as the substrate in 0.1 and 0.001 M perchloric acid solutions, in an acetic acid buffer of pH = 5 and in a biphosphate buffer of pH = 7, for *n*-butyl benzoylformate as the substrate in 1 M

(12) Ciganek, E. *J. Org. Chem.* **1970**, *35*, 862−864.

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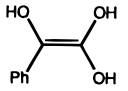
(15) Eisenberg, D.; Kauzmann, W. *The Structure and Properties of Water*; Oxford University Press: New York, 1969; pp 182−187.

hydrochloric acid and in 0.01 M perchloric acid, and for phenyldiazoacetic acid[16] as the substrate in acetic acid buffers of pH ≅ 5 and in 0.02 M sodium hydroxide. Mandelic acid was found to be the principal product in all cases; no phenylglyoxal was formed from the benzoylformate ester substrates, and no acetylmandelic acid was formed from phenyldiazoacetic acid in acetic acid buffers.

Flash Photolysis: Enol Generation. Flash photolysis of either the methyl or butyl esters of benzoylformic acid (**3**) or phenyldiazoacetic acid (**4**) in aqueous solution produced a transient species that absorbed strongly at $\lambda = 270-290$ nm in acidic solutions and at $\lambda = 320-340$ nm in basic solutions. The styrene-type chromophore of mandelic acid enol, **1**, can be expected to have an absorption based in this region—the



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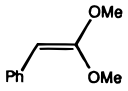

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closely related β,β-dimethoxystyrene, **7**, for example absorbs at $\lambda = 268$ nm[17]—and a shift to longer wavelength in going from acidic to basic solution, in which the enol will be ionized to enolate ion (*vide infra*), is characteristic of the conversion of enols to enolate ions.[18] That, plus the fact that flash photolysis of benzoylformate esters is known to produce phenylhydroxyketene,[9] and ketenes are hydrated rapidly to carboxylic acid enols,[19] suggests that this transient is the enol of mandel acid. This suggestion is confirmed by the form of acid−base catalysis and the solvent isotope effects shown by the decay of this transition (*vide infra*).

Both the appearance and the decay of this transient species conformed to the first-order rate law well. When the appearance and decay were widely separated in time, the data for appearance and decay were analyzed separately, using least-squares fitting of single exponential functions, and when the two rates were similar, fitting was done using a double exponential expression. Rate measurements were made in aqueous solution over the range of acidity $[H^+] = 0.1-10^{-11}$ M, using perchloric acid and sodium hydroxide solutions as well as formic acid, acetic acid, biphosphate ion, hydrogen *tert*-butylphosphonate ion, and tris(hydroxymethyl)methylammonium ion buffers. The ionic strength of the reaction solutions was maintained at 0.10 M.

The benzoylformate ester substrates underwent thermal hydrolysis under strongly basic conditions and were consequently not used above pH = 11, and the phenyldiazoacetic acid substrate underwent thermal hydrolysis in strongly acidic solutions and was not used below pH = 3.

The rate data for appearance of the enol transient are summarized in Tables S1−S6.[20] The measurements in buffers were performed in series of solutions of constant buffer ratio but varying buffer concentration; hydrogen ion concentrations therefore remained constant within a given solution series. Observed first-order rate constants increased linearly with buffer concentration, and extrapolation of the data to zero buffer concentration gave rate constants that represent reaction through catalysis by solvent-derived species only. These rate constants, together with those measured in perchloric acid and sodium hydroxide solutions, are displayed as the upper rate profiles of Figure 1. Hydrogen ion concentrations of the buffer solutions needed to construct this profile were obtained by calculation, using literature acidity constants of the buffer acids and acitivity coefficients recommended by Bates.[21]

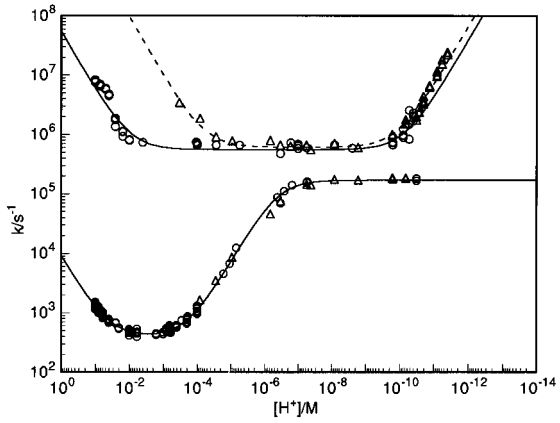


Figure 1. Rate profiles for the generation (upper lines) and ketonization (lower line) of mandelic acid enol, using benzoylformic acid esters (○) and phenyldiazoacetate ion (△) as flash photolysis substrates.

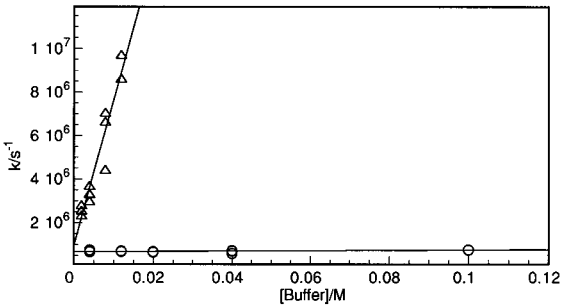


Figure 2. Comparison of rates of formation of mandelic acid enol in acetic acid buffer solutions, buffer ratio = 1, using *n*-butyl benozylformate (○) and phenyldiazoacetic acid (△) as the flash photolysis substrates.

It may be seen that the rate profile obtained using benozylformic acid esters as flash photolysis substrates (Figure 1, circles) is hardly different from that obtained using phenyldiazoacetic acid as the substrate (Figure 1, triangles) in neutral and basic solutions. There is, however, a decided difference in acidic solutions. Least squares analysis of the data gives the following results, for the ester and diazoacid substrates respectively, $k_H{}^+ = (5.45 \pm 0.53) \times 10^7$ M^{-1} s^{-1} and $(9.70 \pm 1.85) \times 10^9$ M^{-1} s^{-1}, $k_o = (5.51 \pm 0.43) \times 10^5$ s^{-1} and $(6.06 \pm 0.44) \times 10^5$ s^{-1}, and $k_{HO}{}^- = (2.33 \pm 0.43) \times 10^9$ M^{-1} s^{-1} and $(4.00 \pm 0.21) \times 10^9$ M^{-1} s^{-1}.

A further difference between the two kinds of substrate is shown by their behavior in buffer solutions. As is illustrated in Figure 2, whereas generation of the enol using phenyldiazoacetic acid as the flash photolysis substrate is strongly catalyzed by acetic acid buffers, that using benzoylformate ester substrates is hardly affected at all by changes in buffer concentration. Least squares analysis of the data shown in Figure 2 gives $k_{obs}/s^{-1} = (9.00 \pm 4.24) \times 10^5 + (6.67 \pm 0.62) \times 10^8$ [buffer] for phenyldiazoacetic acid as the substrate and $k_{obs}/s^{-1} = (6.64 \pm 0.14) \times 10^5 + (8.06 \pm 2.66) \times 10^5$ [buffer] for *n*-butyl benzoylformate as the substrate.

These differences in behavior indicate that the two different kinds of substrate, though they both produce mandelic acid enol (*vide infra*), do this by different reactions through different enol

(16) The pK_a of this acid is 3.70,[13] and it was consequently present as the phenyldiazoacetate ion in most of the studies performed here.

(17) Okuyama, T.; Kawao, S.; Fueno, T. *J. Org. Chem.* **1981**, *46*, 4372−4375.

(18) Haspra, P.; Sutter, A.; Wirz, J. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 617−619.

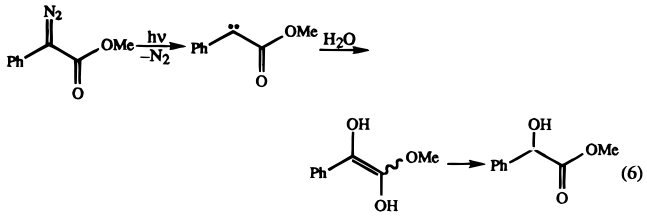
(19) Tidwell, T. T. *Ketenes*; Wiley Interscience: New York, 1995; pp 576−585.

(20) Supporting Information: see paragraph at the end of this paper regarding availability.

(21) Bates, R. G. *Determination of pH Theory and Practice*; Wiley: New York, 1973; p 49.

precursors. The rate profile given by the benzoylformic acid ester substrates, showing a broad region of uncatalyzed reaction and only weak acid and base catalysis, is typical of ketene hydrations.[7a,d,f,h,22] Ketenes, moreover, react only weakly with buffers such as those used here,[23] and they also give only weak solvent isotope effects such as we found by comparing rates of enol formation from *n*-butyl benzoylformate in H_2O and D_2O solution (Table S2[20]): $k_{H_2O}/k_{D_2O} = 1.49 \pm 0.22$.[7d,22b,24] Furthermore, the intensity of the transient signal given by the enol dropped markedly in the region of acid-catalyzed ketene hydration, consistent with the fact that, whereas uncatalyzed and hydroxide-ion catalyzed ketene hydrations occur by a mechanism involving enol intermediates,[19] the acid-catalyzed reaction takes place by a different route that avoids enols.[22d,24] This behavior, plus the fact that flash photolysis of benzoylformate esters is known to produce phenylhydroxyketene,[9] makes it safe to conclude that mandelic acid enol was generated from this ketene in the present study by the hydration reaction shown in eq 4.

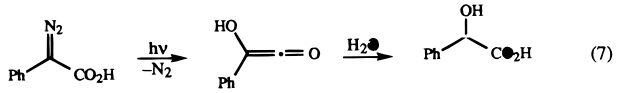
It seems likely, on the other hand, that the different enol precursor formed when phenyldiazoacetic acid is the flash photolysis substrate is phenylcarboxycarbene, **6**, as shown in eq 5. It is well-known that irradiation of aliphatic diazo compounds with light produces carbenes.[25] The overall conversion of phenylcarboxycarbene into mandelic acid, moreover, corresponds to insertion of a carbene into an O−H bond of water, which is also a well-known reaction.[26] We have, in fact, observed a process similar to that of eq 5 upon flash photolysis of methyl phenyldiazoacetate, eq 6,[27] and also of its cyclic



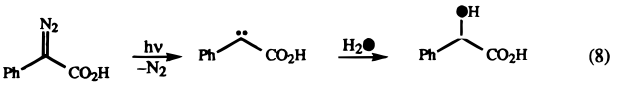
analog, 4-diazo-3-isochromanone.[28] The fact that we observe enol intermediates in these reactions shows that they do not simply involve water and the carbenic carbon atom but that they might be more accurately described as conjugate additon of water across the entire carbonylcarbene function.

These differences in kinetic behavior require that most of the mandelic acid enol formed by flash photolysis of phenyldiazoacetic acid is generated from an intermediate that is not phenylhydroxyketene. This is supported by the oxygen-18 tracer study described below, but the tracer study also suggests that a minor amount of enol obtained from that substrate does arise via the ketene and that some Wolff-rearrangement does in fact take place.

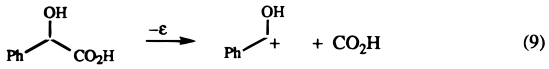
Oxygen-18 Tracer Study. A photo-Wolff reaction[29] of phenyldiazoacetic acid would produce phenylhydroxyketene, whose hydration in oxygen-18 labeled water would give mandelic acid with the isotopic label in its carboxylic acid group, eq 7. On the other hand, hydration of phenylcarboxycarbene,



generated by photoinduced loss of nitrogen from phenyldiazoacetic acid in oxygen-18 labeled water, would give mandelic acid with the isotopic label in its hydroxyl group, eq 8.



We investigated this difference by photolyzing (Rayonet, $\lambda = 254$ nm) 0.01 M phenyldiazoacetic acid in oxygen-18 labeled water to which an equal volume of tetrahydrofuran had been added in order to produce a homogeneous solution. Isotopic analysis of the mandelic acid produced was performed by mass spectroscopy, taking advantage of the fact that mandelic acid fragments readily upon electron impact producing the phenylhydroxymethyl cation as the principal species in its mass spectrum, eq 9.[30] Results obtained in 0.01 M hydrochloric acid



solution showed 96% of the mandelic acid obtained to be isotopically labeled in the hydroxyl group and 4% to be unlabeled at that position, and results obtained in 0.01 M sodium hydroxide solution showed 80% of the mandelic acid labeled in the hydroxyl group and 20% unlabeled at that position.

These results support the formation of phenylcarboxycarbene as the enol precursor produced by flash photolysis of phenyldiazoacetic acid, and they show that this carbene is the principal precursor produced from this substrate. They suggest as well, however, that a minor amount of phenylhydoxyketene is also formed and that some Wolff-rearrangement of phenyldiazoacetic acid does in fact take place.

Flash Photolysis: Enol Ketonization. Rates of ketonization of mandelic acid enol were measured in aqueous perchloric acid and sodium hydroxide solutions and in formic acid, acetic acid, biphosphate ion, hydrogen *tert*-butylphosphonate ion, and tris(hydroxymethyl)methylammonium ion buffers. The data so obtained are summarized in Tables S7−S9.[20]

The measurements in buffers were once again performed in series of solutions of constant buffer ratio and constant ionic strength (0.10 M) but varying buffer concentration, and observed first-order rate constants were again linearly proportional to buffer concentration. Extrapolation of the data to zero buffer concentration then gave rate constants which, together with the values measured in perchloric acid and sodium hydroxde solution, were used to construct the lower rate profile shown in Figure 1.

The circles in this rate profile represent rate constants obtained using benzoylformic acid esters (methyl, *n*-butyl, and isobutyl) as flash photolysis substrates, and the triangles represent those obtained using phenyldiazoacetic acid as the substrate. It may be seen that there is no difference between the two sets of data

(22) (a) Bothe, E.; Dessouki, A. M.; Schulte-Frohlinde, D. *J. Phys. Chem.* **1980**, *84*, 3270−3272. (b) Allen, A. D.; Kresge, A. J.; Schepp, N. P.; Tidwell, T. T. *Can. J. Chem.* **1987**, *65*, 1719−1723. (c) Allen, A. D.; Tidwell, T. T. *J. Am. Chem. Soc.* **1987**, *109*, 2774−2780. (d) Allen, A. D.; Stevenson, A.; Tidwell, T. T. *J. Org. Chem.* **1989**, *54*, 2843−2848. (e) Chiang, Y.; Kresge, A. J.; Popik, V. V. *J. Am. Chem. Soc.* **1995**, *117*, 9165−9171. Chiang, Y.; Guo, H.-X.; Kresge, A. J.; Tee, O. S. *J. Am. Chem. Soc.* **1996**, *118*, 3386−3391.

(23) Andraos, J.; Kresge, A. J. *J. Am. Chem. Soc.* **1992**, *114*, 5643−5646.

(24) Andraos, J.; Kresge, A. J. *J. Photochem. Photobiol. A* **1991**, *57*, 165−173.

(25) Regitz, M.; Maas, G. *Diazo Compounds Properties and Synthes*is; Academic Press: New York, 1986; Chapter 4.

(26) Kirmse, W. *Carbene Chemistry*, 2nd ed.; Academic Press: New York, 1971; pp 423−430.

(27) Chiang, Y.; Kresge, A. J.; Pruszynski, P.; Schepp, N. P.; Wirz, J. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1366−1368.

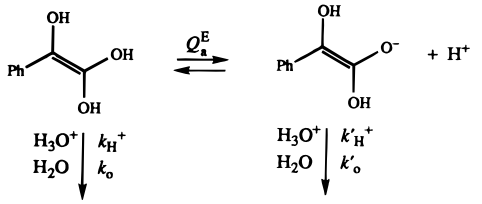
(28) Jefferson, E. A.; Kresge, A. J. Unpublished work.

(29) Regitz, M.; Maas, G. *Diazo Compounds Properties and Synthesis*; Academic Press: New York, 1986; pp 185−195.

(30) Sharp, R. T. *Org. Mass Spec.* **1980**, *15*, 381−382.

and that both kinds of substrate are producing the same transient species. This similarity is corroborated by the buffer-dependent portions of observed rate constants, as is illustrated in Figure 3. This figure shows that there is no significant difference between rate constants measured in biphosphate buffers using methyl benzoylformate, isobutylbenzoylformate, and phenyldiazoacetic acid as the flash photolysis substrates, which stands in marked contrast to the different behavior shown by ester and diazoacid substrates illustrated in Figure 2. Least squares analysis of the data shown in Figure 3 gives $k_{obs}/s^{-1} = (1.61 \pm 0.04) \times 10^5 + (3.21 \pm 0.22) \times 10^6$ [buffer] for methyl benzoylformate as the substrate, $k_{obs}/s^{-1} = (1.55 \pm 0.04) \times 10^5 + (3.80 \pm 0.20) \times 10^6$ [buffer] for isobutyl benzoylformate as the substrate, and $k_{obs}/s^{-1} = (1.43 \pm 0.02) \times 10^5 + (3.68 \pm 0.10) \times 10^6$ [buffer] for phenyldiazoacetic acid as the substrate. This similarity, of course, is the behavior expected on the basis of the reaction schemes of eqs 4 and 5.

The lower rate profile of Figure 1 is typical of enol ketonization reactions,[5] and it may be interpreted in the usual way in terms of reaction through rate-determining β-carbon protonation of either enol or enolate ion and either hydronium ion or water as the proton source, as shown in eq, 10.[31] The diagonal region of acid catalysis at the high acidity end of this rate profile then represents ketonization of un-ionized enol with



(10)

hydronium ion acting as the protonating agent. The short portion of apparently uncatalyzed reaction at $[H^+] = 10^{-2}$–10^{-3} M immediately following this diagonal region could represent either un-ionized enol reacting with water as the proton source or ionization of the enol to the much more reactive enolate ion followed by carbon protonation of that by hydronium ion. It is sometimes possible to exclude the second of these two possibilities because the data require a rate constant for the carbon protonation step that is impossibly large, but that is not the case here (*vide infra*). In the second diagonal region of the rate profile following this uncatalyzed reaction, ketonization still begins with un-ionized enol as the initial state, but ionization to the more reactive enolate ion is followed by carbon protonation with water as the proton source; since the concentration of enolate ion under these conditions is inversely proportional to $[H^+]$ and hydroniuum ion is not involved in the rate-determining step, this produces an apparent hydroxide-ion catalysis. At sufficiently low acidities, where the enol ionization equilibrium shifts over to enolate ion, this hydroxide-ion catalysis becomes saturated, and the second region of uncatalyzed reaction above $[H^+] = 10^{-7}$ M results. This saturation of hydroxide-ion catalysis produces a bend in the rate profile that corresponds to the acidity constant of the enol.

The rate law that applies to the reaction scheme of eq 10 is shown as eq 11. The rate constants in this expression are as

$$k_{obs} = k_H{}^+[H^+] + k_{uc} + k'_o Q_a^E/(Q_a^E + [H^+]) \qquad (11)$$

defined in eq 10, except for that representing the uncatalyzed

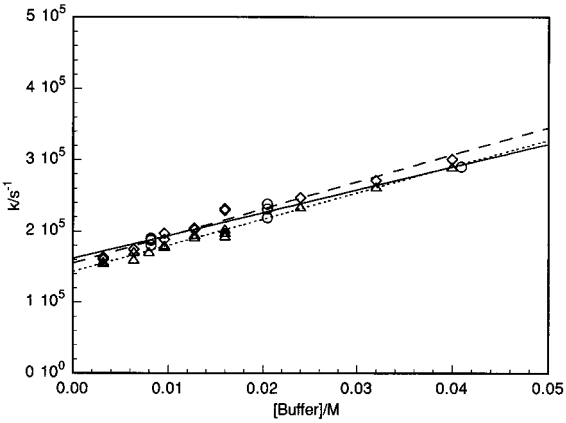


Figure 3. Comparison of rates of ketonization of mandelic acid enol in biphosphate ion buffer solutions, buffer ratio = 1/3, using methyl benzoylformate (○), isobutyl benzoylformate (◇), and phenyldiazoacetic acid (△) as the flash photolysis substrates.

reaction at $[H^+] = 10^{-2}$–10^{-3} M, which, because of the ambiguity regarding reaction mechanism in this region, is simply designated k_{uc}. Least squares fitting of the data using this expression produced the following results: $k_H{}^+ = (9.03 \pm 0.27) \times 10^3$ M^{-1} s^{-1}, $k_{uc} = (3.89 \pm 0.08) \times 10^2$ s^{-1}, $k'_o = (1.70 \pm 0.04) \times 10^5$ s^{-1}, and $Q_a^E = (4.12 \pm 0.18) \times 10^{-7}$ M, pQ_a^E = 6.39 ± 0.02.[33]

As was pointed out above, the process represented by k_{uc} in the rate law of eq 11 could be either carbon protonation of un-ionized enol by water or ionization of the enol to enolate ion followed by carbon protonation of enolate by hydronium ion. In the latter case, this part of the rate law becomes $k'_H{}^+ Q_a^E$ with $k'_H{}^+$ being the rate constant for carbon protonation of the enolate ion; least squares analysis then gives $k'_H{}^+ = (8.62 \pm 0.47) \times 10^8$ M^{-1} s^{-1}, with $k_H{}^+$, k'_o, and Q_a^E as listed above. This value of $k'_H{}^+$, though large, does not exceed the diffusion-controlled limit, and it consequently cannot be used to rule out this reaction path.

Support for interpretation of the process we observe as the ketonization of mandelic acid enol according to the reaction scheme of eq 10 comes from its behavior in buffer solutions. As Figure 3 illustrates, rates of reaction increase with increasing buffer concentration. The nature of this buffer catalysis was investigated with the aid of eq 12, in which k_{cat} is the slope of a buffer dilution plot ($\Delta k_{obs}/\Delta$[buffer]),[34] k_B and k_{HA} are general

$$k_{cat} = k_B + (k_{HA} - k_B) f_A \qquad (12)$$

base and general acid catalytic coefficients respectively, and f_A is the fraction of buffer present in the acid form. Application of this equation showed general base catalysis in formic and acetic acid buffers and general acid catalysis in biphosphate ion buffers. This behavior is consistent with the fact that the measurements in formic and acetic acid buffers were made in the region of the rate profile showing base catalysis, where reaction is expected to take place by conversion of the enol to the more reactive enolate ion followed by rate-determining carbon protonation of the latter, eq 13.

The rate-determining carbon protonation step of such a reaction sequence will, of course, show general acid catalysis,

(31) We have written the ionization of mandelic acid enol as involving the hydroxyl groups *trans* to phenyl because the *trans* enol of phenylacetaldehyde is a stronger acid than the *cis* isomer.[32] Ionization of the hydroxyl group geminal to phenyl can be ruled out because ketonization would then produce phenylglyoxal, which is not found as a reaction product.

(32) Chiang, Y.; Kresge, A. J.; Walsh, P. A.; Yin, Y. *J. Chem. Soc., Chem. Commun.* **1989**, 869−871.

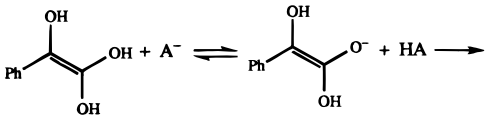
(33) This is a *concentration* dissociation constant, applicable at ionic strength = 0.10 M.

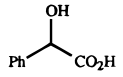
(34) The slopes of buffer dilution plots for some acetic acid and biphosphate ion buffers were adjusted to compensate for the fact that the enol existed in both ionized and un-ionized forms in these solutions.

Table 1. General Acid Catalytic Coefficients for Rate-Determining Carbon Protonation of Mandelic Acid Enolate Ion[a]

acid	pK_a	$k'_{HA}/10^6$ M^{-1} s^{-1}
HCO_2H	3.75	58.6
CH_3CO_2H	4.76	33.5
$H_2PO_4^-$	7.20	14.7
$(CH_2OH)_3CNH_3^+$	8.07	8.96
t-$BuPO_3H^-$	8.71	4.80

[a] Ionic strength = 0.10 M.





(13)

but this will be converted into general base catalysis for the overall process by the action of base upon the enol in the prior equilibrium. The biphosphate ion buffers, on the other hand, were used in a region of the rate profile beyond the acidity constant of the enol where the enol was converted to enolate ion. Reaction here is expected to take place by simple carbon protonation of the enolate substrate, *i.e.*, by the last step of the sequence shown in eq 13, which will give general acid catalysis. Thus, both the fact that buffer catalysis is observed and the nature of this catalysis are wholly consistent with the identification of this reaciton as ketonziation of mandelic acid enol.

Rate measurements in hydrogen *tert*-butylphosphonic acid and tris(hydroxymethyl)methyl ammonium ion buffers were performed at only one buffer ratio and eq 12 could therefore not be applied. In these solutions, however, as in the biphosphate ion buffers, the enol was converted to enolate ion, and it seems safe to assume that general acid catalysis was operating here as well. The data were therefore treated accordingly.

The general base catalytic coefficients for formic and acetic acids could be converted into general acid coefficients for rate-determining carbon protonation of the enolate ion by using the relationship shown in eq 14, in which Q_a^{HA} is the acidity constant of the catalyzing acid.[33] The

$$k_B = k'_{HA}Q_a^E/Q_a^{HA} \qquad (14)$$

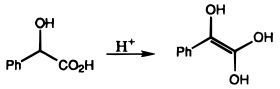
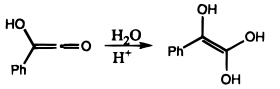
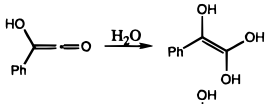
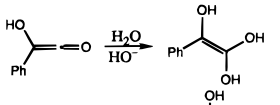
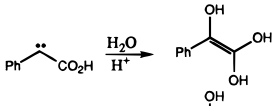
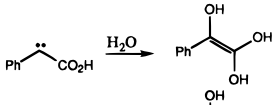
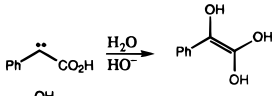
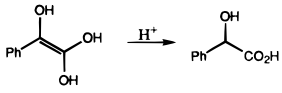
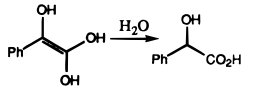
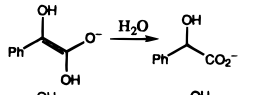
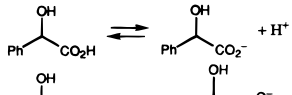
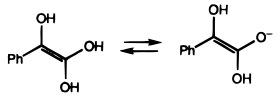
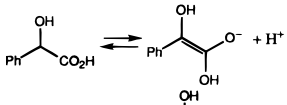
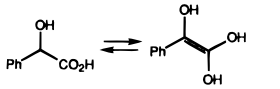
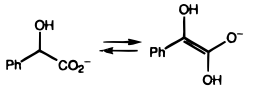
results so obtained, together with the directly determined values for the other buffer acids, are listed in Table 1. It may be seen that these catalytic coefficients form a sensible series, decreasing in value with decreasing acid strength of the catalyst. They do, in fact, give a reasonably good Brønsted relation, shown in Figure 4, despite the fact that the catalysts they represent vary considerably in charge type. The exponents of Brønsted relations are believed to provide a measure of transition state structure,[35] and the low value produced in the present case, $\alpha = 0.24 \pm 0.01$, implies an early, reactant-like transition state, which is consistent with the fact that the present reactions are quite rapid.[36]

Further support for the identification of the present reaction as the ketonization of mandelic acid enol comes from isotope effects based upon rate measurements made in D_2O solutions of perchloric acid and sodium hydroxide. The data are summarized in Tables S7 and S8, and the results in perchloric acid are compared with those for H_2O solutions of this acid in Figure 5.

(35) Kresge, A. J. In *Proton Transfer Reactions*; Caldin, E. F., Gold, V., Eds.; Chapman and Hall: London, 1975; Chapter 7.

(36) Hammond, G. S. *J. Am. Chem. Soc.* **1955**, *77*, 334−338.

Table 2. Summary of Rate and Equilibrium Constants[a]

Process	Constant
	$k_{H^+} = 5.85\times10^{-13}$ M^{-1} s^{-1}
	$k_{H^+} = 5.45\times10^{7}$ M^{-1} s^{-1}
	$k_o = 5.51\times10^{5}$ s^{-1}; $(k_o)_{H2O}/(k_o)_{D2O} = 1.49$
	$k_{HO^-} = 2.33\times10^{9}$ M^{-1} s^{-1}
	$k_{H^+} = 9.70\times10^{9}$ M^{-1} s^{-1}
	$k_o = 6.06\times10^{5}$ s^{-1}
	$k_{HO^-} = 4.00\times10^{9}$ M^{-1} s^{-1}
	$k_{H^+} = 9.03\times10^{3}$ M^{-1} s^{-1}; $k_{H^+}/k_{D^+} = 3.25$
	$k_{uc} = 3.89\times10^{2}$s^{-1}; $(k_{uc})_{H2O}/(k_{uc})_{D2O} = 5.31$
	$k'_o = 1.70\times10^{5}$ s^{-1}
	$Q_a = 5.86\times10^{-4}$ M; p$Q_a = 3.23$
	$Q_a^E = 4.12\times10^{-7}$ M; p$Q_a^E = 6.39$
	$Q_a^K = 2.67\times10^{-23}$ M; p$Q_a^K = 22.57$
	$K_E = 6.47\times10^{-17}$; p$K_E = 16.19$
	$K'_E = 4.55\times10^{-20}$; p$K'_E = 19.34$

[a] 25 °C; ionic strength = 0.10 M.

This figure shows that rates of reaction are slower in D_2O than in H_2O throughout the range of acidity investigated but that the difference is greater in the region of apparent hydroxide ion catalysis at low acidity than in the region of acid catalysis at higher acidity. This is the behavior expected on the basis of the reaction scheme of eq 10, for, whereas the isotope effect in the region of acid catalysis is on a rate constant alone (k_{H^+}), that in the region of base catalysis is on the product of a different rate constnt (k'_o) and an equilibrium constant (Q_a^E). Least squares analysis of the D_2O data gave results that, when combined with H_2O values, produced the following isotope effects: $k_{H^+}/k_{D^+} = 3.25 \pm 0.12$ and $(k'_oQ_a^E)_{H2O}/(k'_oQ_a^E)_{D2O} = 30.8 \pm 2.9$. The latter could be separated into its constituent parts with the aid of $(k'_o)_{H2O}/(k'_o)_{D2O} = 6.90 \pm 0.54$ based upon the measurements made in sodium hydroxide solutions; the result is $(Q_a^E)_{H2O}/(Q_a^E)_{D2O} = 4.47 \pm 0.55$.

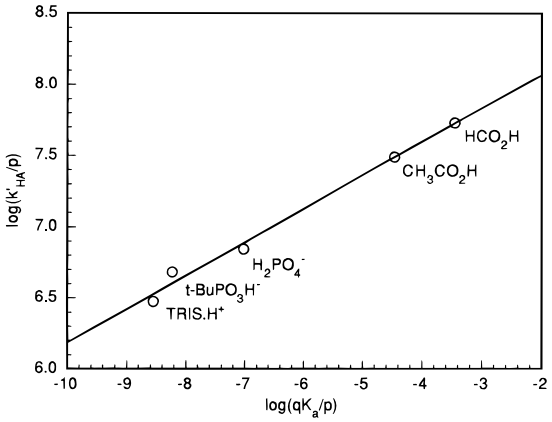


Figure 4. Brønsted plot for the ketonization of mandelic acid enol.

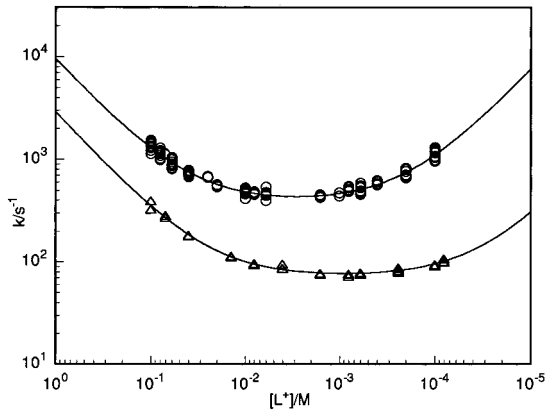


Figure 5. Comparison of rates of ketonization of mandelic acid enol in H_2O (○) and D_2O (△) solutions of perchloric acid.

The first of these isotope effects is a typcial value for rate-determining hydron transfer from the hydronium ion,[37] and similar results have been obtained for ketonization of other enols.[5] This kind of isotope effect actually consists of a primary component in the normal direction, $k_H/k_D > 1$, plus an offsetting secondary component in the inverse direction, $k_H/k_D < 1$, produced by changes in the nonreacting bonds of the hydronium ion.[37] Isotope effects on hydron transfer from a water molecule also contain a secondary component in addition to a normal primary component, but now the secondary component is in the normal direction and consequently augments the primary effect. It is significant, therefore, that the isotope effect observed here on k'_o, which according to the scheme of eq 10 represents protonation of enolate ion by a water molecule, is greater than that on k_H^+. The isotope effect on Q_a^E is as expected as well: isotope effect theory requires oxgyen acids to be more completely ionized in H_2O than in D_2O,[37b] and the value observed here is typical for acids of the strength of the present enol.[38]

Enolization. In the early stages of the present investigation, we measured rates of acid-catalyzed racemization of mandelic acid, and, in the belief that racemization was occurring by an enolization pathway, we used these rate constants to determine the keto−enol equilibrium constant reported in a preliminary publication of this work.[7b] These data are summarized in Table S10.[20]

We later discovered, however, that acid-catalyzed deuterium incorporation from D_2O into the α-position of mandelic acid, a more reliable but operationally more difficult measure of enolization, takes place several times more slowly than racemization. Since the acid-catalyzed enolization of carbonyl compounds gives inverse solvent isotope effects and is consequently faster in D_2O than in H_2O,[5] deuterium incorporation should have been faster rather than slower than racemization, if both reactions were indeed taking place by an enolization pathway. Racemization of mandelic acid, of course, could occur by another pathway, *e.g.*, by protonation of its α-hydroxyl group followed by displacement of that by a water molecule. It is difficult, on the other hand, to conceive of a reasonable nonenolization pathway for α-hydrogen exchange. We conclude, therefore, that acid-catalyzed racemization does not occur via enolization, and we now use deuterium incorporation to monitor enolization.

Deuterium incorporation into the α-position of mandelic acid is a very slow process, and concentrated acid solutions and high temperatures had to be used to obtain reasonable reaction rates. Rate measurements were made in 4 M DCl/D_2O solutions at five temperatures in the range 130−150 °C, and at two of these temperatures (140 and 150 °C) measurements were also made over a range of acidities, from 4 M down to 0.5 M. These data are summarized in Table S11.[20]

As is commonly the case for reactions conducted in concentrated acid solutions, observed first-order rate constants increased with acidity more rapidly than in direct proportion to acid concentration; an acidity function was therefore used to extrapolate the data down to dilute solution, in order to obtain the second-order hydronium-ion catalytic coefficient needed for calculating a keto−enol equilibrium constant. The Cox−Yates technique using X functions[39] appears to be the best method currently available for this purpose.[40] The X scale has not been determined for DCl/D_2O solutions, but the values of other acidity functions have been found not to change in going from H_2O to D_2O when the comparison is made at the same acid molarity,[41] and the X scale for HCl/H_2O[42] was consequently used instead. Values of X at the temperatures of the kinetic measurements were calculated from 25 °C values using the relationship given in eq 15.[43] The correlation function employed was the standard Cox−Yates expression, shown as eq 16, in which k_D^+ is the desired dilute solution rate constant and m is a slope parameter. When X is adjusted for temperature differences according to

$$X_T = X_{25}[298.15/(273.15 + T°C)] \quad (15)$$

$$\log(k_{obs}/[D^+]) = \log k_H^+ + mX_T \quad (16)$$

eq 15, m is expected to be temperature invariant.[43] This was found to be the case in the present work for the two temperatures at which rates were measured over a range of acid concentrations: $m = 0.61 \pm 0.03$ at 139.6 °C and $m = 0.69 \pm 0.03$ at 151.2 °C. The average of these two values was therefore used to extrapolate down to dilute solution from the rate constants measured in 4 M acid at all temperatures.

The dilute solution hydronium-ion catalytic coefficients obtained in this way gave a good linear Eyring plot, shown in Figure 6. Least squares analysis produced the activation parameters $\Delta H^‡ = 30.9 \pm 0.3$ kcal mol^{-1} and $\Delta S^‡ = -9.4 \pm$

(37) (a) Kresge, A. J.; Sagatys, D. S.; Chen, H. L. *J. Am. Chem. Soc.* **1977**, *99*, 7228−7233. (b) Kresge, A. J.; More O'Ferrall, R. A.; Powell, M. F. In *Isotopes in Organic Chemistry*; Buncel, E., Lee, C. C., Eds.; Elsevier: Amsterdam, 1987; Vol. 7, pp 177−273.

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(39) Cox, R. A.; Yates, K. *J. Am. Chem. Soc.* **1978**, *100*, 3861−3867.

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(41) Högfeldt, E.; Bigeleisen, *J. Am. Chem. Soc.* **1960**, *82*, 15−20. Cox, R. A.; Lam, S.-O.; McClelland, R. A.; Tidwell, T. T. *J. Chem. Soc., Perkin Trans. II* **1979**, 272−275.

(42) Cox, R. A.; Yates, K. *Can. J. Chem.* **1981**, *59*, 2116−2124.

(43) Cox, R. A.; Goldman, M. F.; Yates, K. *Can. J. Chem.* **1979**, *57*, 2960−2966.

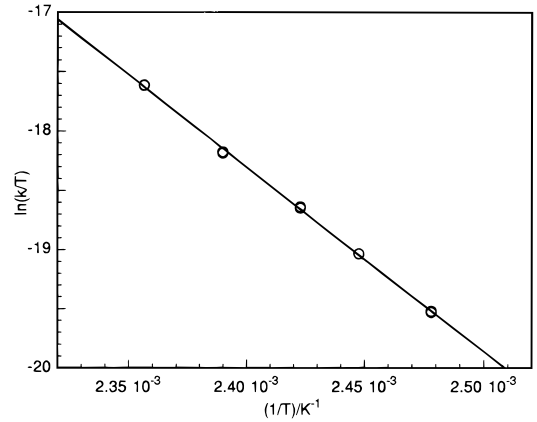


Figure 6. Eyring plot for the acid-catalyzed incorporation of deuterium into the α-position of mandelic acid.

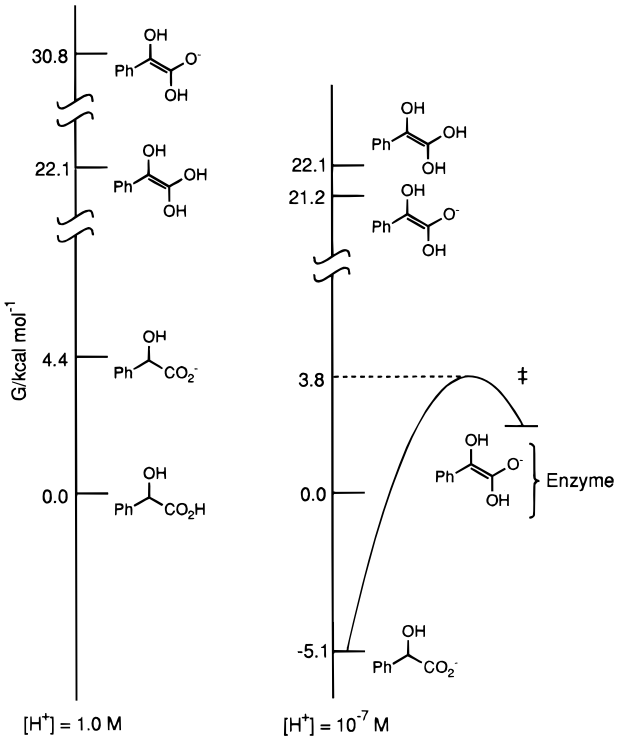


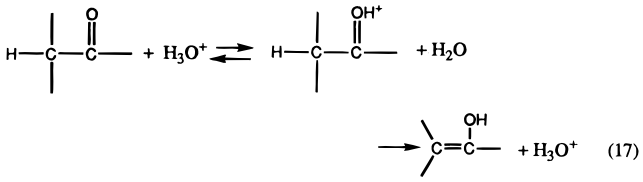
Figure 7. Free energy diagram comparing the mandelic acid keto−enol system to racemization catalzyed by mandelate racemase.

0.8 cal K^{-1} mol^{-1}, and extrapolation down to 25 °C gave the rate constant $k_{25} = (1.17 \pm 0.18) \times 10^{-12}$ M^{-1} s^{-1}.

The rate constant $k_D{}^+ = (3 \pm 1) \times 10^{-5}$ M^{-1} s^{-1} has recently been reported for acid-catalyzed deuterium incorporation into mandelic acid at 170 °C.[44] Extrapolation of the present data to this temperature gives $k_D{}^+ = (4.6 \pm 0.1) \times 10^{-5}$ M^{-1} s^{-1}, consistent with this reported value.

Discussion

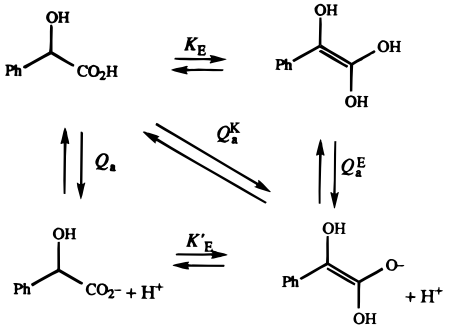
Keto−Enol and Related Equilibria. An estimate of the keto−enol equilibrium constant for mandelic acid may be made by combining the rate constants for hydronium-ion-catalyzed enolization and ketonization determined here. Allowance must be made, however, for the fact that the enolization rates were measured in D_2O, where D_3O^+ was the catalyst, whereas the ketonization rates were measured in H_2O, where H_3O^+ was the catalyst.[45] The well established mechanism for hydronium-ion-catalyzed enolization of carbonyl compounds[5] consists of equilibrium protonation on carbonyl carbon followed by rate-determining removal of α-hydrogen by a water molecule, eq 17. Conversion of the relatively loose bonds of the hydronium



(17)

ion into the tighter bonds of a water molecule in the equilibrium step of this reaction sequence produces an inverse solvent isotope effect whose magnitude is usually, *ca*. 0.5; for example, $k_H{}^+/k_D{}^+ = 0.57$ for the enolization of acetaldehyde,[3] $k_H{}^+/k_D{}^+ = 0.46-0.54$ for enolization of acetone,[46] and $k_H{}^+/k_D{}^+ = 0.56$ for enolization of isobutyrophenone.[47] No determination of this isotope effect for the enolization of a carboxylic acid appears to have been made, and, in the absence of more definite information, we shall use the nominal value $k_H{}^+/k_D{}^+ = 0.5$ to convert the rate constant for deuterium incorporation in D_2O into a rate constant for enolization in H_2O: $k^E_{H^+} = 0.5$ $(1.17 \pm 0.18) \times 10^{-12} = (5.85 \pm 0.91) \times 10^{-13}$ M^{-1} s^{-1}. Combination of this result with the rate constant for hydronium-ion-catalyzed ketonization then leads to the keto−enol equilibrium constant $K_E = k^E_{H^+}/k^K_{H^+} = (5.85 \pm 0.91) \times 10^{-13}/(9.07 \pm 0.31) \times 10^3 = (6.48 \pm 1.02) \times 10^{-17}$, $pK_E = 16.19 \pm 0.07$. The error limit cited here is a statistical uncertainty (standard deviation) which does not include possible systematic errors; allowance for that, plus the uncertainty in the estimated solvent isotope effect, leads to the probably more realistic assessment $pK_E = 16.2 \pm 0.2$. The latter estimate is different, by a factor of *ca*. 6, from the result, $pK_E = 15.4$, reported earlier based upon the misguided assumption that acid-catalyzed racemization of mandelic acid occurs by an enolization pathway.[7b]

As the thermodynamic cycle of eq 18 shows, this equilibrium constant connecting un-ionized keto and enol isomers is related



(18)

to the keto−enol equilibrium constant for ionized species by two acidity constants, that for the carboxyl group of mandelic aicd, Q_a, and that for conversion of enol to enolate ion, Q^E_a.

(44) Bearne, S. L.; Woflenden, R. *Biochemistry* **1997**, *36*, 1646−1656.

(45) The rate constant for ketonization in D_2O with D_3O^+ as the catalyst is also available from the present work. However, comparison of that with the enolization rate constant in D_2O, in order to avoid making an estimate of the solvent isotope effect, involves an unknown primary isotope effect: enolization, *i.e*., deuterium incorporation, was necessarily monitored using α-protiomandelic acid as the substrate; H was consequently the hydrogen in flight in the rate-determining step in these experiments, whereas D was in flight inthe D_3O^+/D_2O ketonization measurements. Primary isotope effects are usually greater and more variable than solvent isotope effects and are consequently more difficult to estimate.

(46) Reitz, O. *Naturwissenschaften* **1936**, *24*, 814−814. Albery, W. J.; Gelles, J. S. *J. Chem. Soc., Faraday Trans. 1* **1982**, *78*, 1569−1578. Shelly, K. P.; Venimadhavan, S.; Nagaranjan, K.; Stewart, R. *Can. J. Chem.* **1989**, *67*, 1274−1282.

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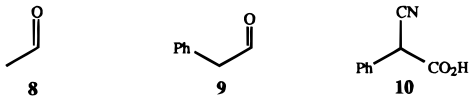
The latter constant has been determined in the present work, and the former may be estimated by applying activity coefficients[21] to a literature value of the thermodynamic acidity constant of mandelic acid.[48] Use of these values then leads to the result $K'_E = (4.55 \pm 0.75) \times 10^{-20}$, $pK'_E = 19.34 \pm 0.07$. The reaction scheme of eq 18 also allows evaluation of the acidity constant of mandelic acid ionizing as a carbon acid, $Q_a^K = (2.67 \pm 0.44) \times 10^{-23}$, $pQ_a^K = 22.57 \pm 0.07$.[33]

Comparison with Other Systems. The keto−enol equilibrium constant for mandelic acid determined here, $pK_E = 16.2$, is many orders of magnitude less than those for simple aldehydes and ketones, *e.g.*, $pK_E = 6.23$ for acetaldehyde[3] and $pK_E = 8.33$ for acetone.[49] This striking difference may be attributed to resonance stabilization of the keto form in the carboxylic acid system, produced by interaction of its hydroxyl and carbonyl moieties; this interaction is lost in the enol, and it consequently serves to increase the energy difference between keto and enol forms, thus making K_E as small as it is.

The value for mandelic acid, on the other hand, is four orders of magnitude greater than the estimte for acetic acid, $pK_E = 20$.[2] It is instructive to compare this difference with the effect of a β-phenyl group on keto−enol equilibrium constants, as assessed by the difference between $pK_E = 6.23$ for acetaldehyde, **8**, and $pK_E = 2.88$ for phenylacetaldehyde, **9**.[32] This difference,

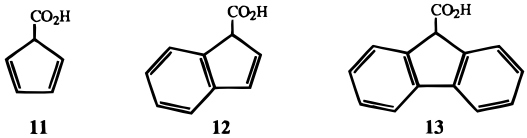


8 **9** **10**

$\Delta pK_E = 3.35$, is not unlike the four pK difference between mandelic and acetic acids, which suggests that most of the increased enol content of mandelic acid is due to the phenyl group and that the β-hydroxyl substituent makes little contribution.

The effect of a β-cyano group, on the other hand, is very much stronger: $pK_E = 7.22$ for cyanophenylacetic acid, **10**.[7e] This very powerful influence of the cyano group is much stronger than its simple double bond stabilizing effect, $D = 2.3$ kcal/ mol^{-1},[8a] and must be due in large part to an additional synergistic interaction of the electronegative cyano group and electropositive enol hydroxyl groups, through the enol double bond. The corresponding interaction of a hydroxyl group in place of cyano is, of course, antagonistic because the groups involved here are all electropositive, and that works against the quite appreciable simple double-bond stabilizing effect of hydroxyl, $D = 5.4$ kcal mol^{-1}.[8b]

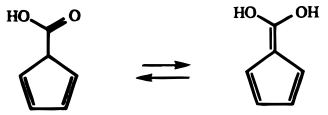
Cyclopentadiene carboxylic acid, **11**, and its monobenzo, **12**, and dibenzo, **13**, analogs also have keto−enol equilibrium constants that are very much greater than the estimate $pK_E = 20$ for acetic acid: for these substances, pK_E lies in the range



11 **12** **13**

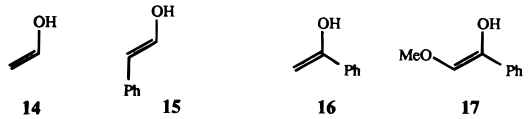
8.4−9.7.[7a,g,i] Formation of the enol in these systems places a double bond exocyclic to a cyclopentadiene ring, eq 19, which creates fulvenoid resonance.

The acidity constant for mandelic acid enol determined here, $pQ_a^E = 6.39$ makes this substance considerably more acidic than enols of simple aldehydes and ketones, *e.g.*, $pQ_a^E = 10.50$



(19)

for the enol of acetaldehyde[3] and $pQ_a^E = 10.94$ for the enol of acetone.[50] Comparison of the value for acetaldedhye enol, **14**, with $pQ_a^E = 9.46$ for the *trans* enol of phenylacetaldehyde,



14 **15** **16** **17**

15,[32] suggests that about one pK unit of the increased acidity of mandelic acid enol is due to the phenyl substituent. The β-hydroxy group of mandelic acid enol, on the other hand, probably has a slight acid-weakening effect, as evidenced by $pQ_a^E = 10.40$ for acetophenone enol, **16**[51] and $pQ_a^E = 10.86$ for the enol of α-methoxyacetophenone, **17**.[52] It seems likely that the major factor influencing the acidity of mandelic acid enol is the presence of a second hydroxyl group geminal to the first: *gem*-diols in saturated systems are *ca*. 2 pK units more acidic than the corresponding alcohols,[53] and this effect of the second hydroxyl might well be transmitted more efficiently through an unsaturated carbon atom and thus be stronger in *gem*-enediols.

The acid strength of mandelic acid enol is similar to that of the enol of methyl mandelate, for which $pQ_a^E = 6.62$,[27] showing that the geminal effects of hydroxyl and methoxyl are similar. The acidity of the enols of cyclopentadiene carboxylic acid and its benzo analogs, **11**−**13**, however, is considerably greater: $pQ_a^E = 1.3-2.0$ for these substances[7a,g,i] and $= 0.4-1.3$ for the enols of some of the ring-sulfonated derivatives of **12**.[7d] The remarkably strong acidity of these substances may be attributed to delocalization of the negative charge of their enolate ions into the cyclopentadiene ring, which creates an aromatic system. The enol of cyanophenylacetic acid, **10**, is also very acidic: $pQ_a^E = 0.99$ for this substance.[7e] The difference between this value and $pQ_a^E = 6.35$ for mandelic acid enol attests to the well-known very strong acidifying effect of the cyano group.

Mandelate Racemase. We have found in the present study that racemization of mandelic acid and deuterium incorporation into its α-position occur at different rates in acid solution and that acid-catalyzed racemization therefore cannot be taking place by an enolization pathway. The situaton is different in neutral and basic solution: in these media racemization and deuterium incorporation do occur at the same rate,[44] which implies that the base-catalyzed racemization that takes place under these conditions does occur by an enolization pathway.

The racemization of mandelic acid in neutral solution is also catalyzed very efficiently by the enzyme mandelate racemase, and recent detailed mechanistic studies[10] have shown that the enzymatic reaction occurs by an enolization mechanism as well. Our results provide a quantitative measure of the thermodynamic barrier that this enzyme must overcome in catalyzing this reaction.

The free energy diagram given in Figure 7 presents our results in graphical form. The scale on the left side of this diagram

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(49) Chiang, Y.; Kresge, A. J.; Schepp, N. P. *J. Am. Chem. Soc.* **1988**, *111*, 3977−3980.
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(51) Jefferson, E. A.; Keeffe, J. R.; Kresge, A. J. *J. Chem. Soc., Perkin Trans. 2* **1995**, 2041−2046.
(52) Kresge, A. J.; Yin, Y. Unpublished work.
(53) Stewart, R. *The Proton: Applications to Organic Chemistry*; Academic Press: New York, 1985; p 44.

shows that mandelic acid enol and enolate ion lie 22.1 and 30.8 kcal mol^{-1}, respectively, above un-ionized mandelic acid. These values refer to the commonly employed standard state [H^+] = 1.0 M. The enzymatic reaction, however, takes place in neutral solution, and switching over to the standard state [H^+] = 10^{-7} M lowers the free energies of the ionized species by $RT \ln(10^7)$ = 9.5 kcal mol^{-1}. This is shown by the scale on the right side of Figure 7, which also reveals that in neutral solution mandelic acid enol and enolate ion have closely similar free energies and therefore differ little in stability; either one of these species could consequently be involved in the enzymatic reaction with equal facility. These species lie an average of 26.8 kcal mol^{-1} above the mandelate ion, which is the form in which the substrate will exist in the neutral solution of the enzymatic reaction. The thermodynamic barrier that the enzyme must overcome in forming the enol or enolate ion intermediates involved in its reaction is thus some 27 kcal mol^{-1}.

This thermodynamic barrier is much greater than the free energy of activation of the enzymatic reaction. Using k_{cat} = 1070 s^{-1} and K_m = 0.63 mM[54] gives a rate constant, referenced to free enzyme and mandelate ion as the initial state, that corresponds to the free energy of activation $\Delta G^{\ddagger}$ = 9.0 kcal mol^{-1}. This puts the free energy of the transition state of the enzymatic reaction some 18 kcal mol^{-1} below that of the enol or enolate ion. The enzyme must therefore stabilize its reaction intermediate by this amount if its transition state lies at the same free energy as the enol or enolate ion intermediate and by somewhat more if, as seems likely, the intermediate lies below the transition state. Two different hypotheses have recently been advanced to account for this stabilization: one attributes it to formation of a strong "low-barrier" hydrogen bond between the intermediate and an essential glutamic acid residue at the active site of the enzyme[11a,55] and another attributes it to electrostatic stabilization of the intermediate by the divalent metal ion cofactor needed by the enzymatic reaction.[11b] Currently available evidence would seem to favor the latter explanation.[56]

Acknowledgment. We are grateful to the Natural Sciences and Engineering Research Council of Canada and the United States National Institutes of Health for financial support of this work.

Supporting Information Available: Tables S1−S11 of rate data (24 pages). See any current masthead page for ordering and Internet access instructions.

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