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Generation and study of enols and other reactive species

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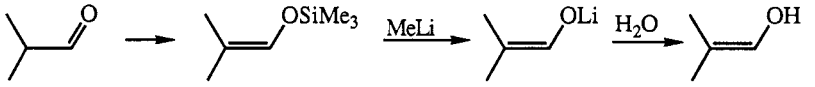
Abstract – A general description of methods recently developed in our laboratory for generating and studying enols and other reactive species is presented, together with an overview of the kinds of substances that have been made and the kind of information that has been obtained. This is then followed by a more detailed discussion of two systems of some biological importance, the enol of pyruvic acid and the enol of mandelic acid. Our results show that the masked enol function in phosphoenolpyruvate contributes half of the free energy liberated in the hydrolysis of this high-energy compound, and that racemization of mandelate ion by mandelate racemase cannot occur by enzymatically promoted enolization followed by release of enol or enolate and reketonization off the enzyme.

INTRODUCTION

The past decade has seen a surge of interest in the chemistry of simple enols, stimulated largely by the development of methods of generating these labile substances under conditions where they can be studied in detail (ref. 1). This, in turn, has led to the investigation of other reactive species, some of which had never been observed before. This lecture will begin with a general description of the methods we have devised in our laboratory for examining reactive species, together with a brief overview of the kinds of substances we have been able to make and the kind of information we have been able to obtain. This will then be followed by a more detailed discussion of what we have learned about two systems of some biological importance: the enol of pyruvic acid and the enol of mandelic acid; the first of these is the enol of a ketone while the second is a carboxylic acid enol.

METHODS OF GENERATION

The first reactive species we studied was the enol of isobutyraldehyde (ref. 2). We generated this substance by hydrolysis of its alkali metal enolate, which itself was made via the trimethylsilyl enol ether using methodology that had been in the literature for some time, eq. 1 (ref. 3):



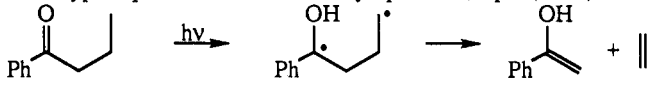
(1)

We found that adding a small quantity of a tetrahydrofuran or dioxane solution of lithium enolate to a large excess of water resulted in an immediate oxygen-to-oxygen proton transfer which produced the enol; the enol then reverted back to its keto isomer at a slower rate. We learned later that we could prepare some alkali-metal enolates directly by treating the carbonyl compound with potassium hydride (ref. 4), thus circumventing synthesis of the trimethylsilyl ether (ref. 2b,5). Sometimes the silyl ether can also be used directly, for hydrolysis of trimethylsilyl enol ethers gives enols (ref. 6), and in some cases this hydrolysis occurs more rapidly than subsequent ketonization of the enol (ref. 2b,7); fluoride ion catalysis of the silyl ether hydrolysis reaction is helpful in making this a useful method.

Ketonization reactions may be monitored readily by ultraviolet spectroscopy. Aliphatic enols have strong absorption bands near $\lambda = 200$ nm where simple aldehydes and ketones do not absorb at all, and ketonization is therefore accompanied by a marked change in uv spectrum. Because this change is strong, kinetic data of high quality can easily be obtained.

These alkali metal and silyl ether methods of generating enols require mixing two solutions, and, because there is a limit to how fast solutions can be mixed, they can be applied only to the less reactive enols, such as that of isobutyraldehyde whose lifetime in aqueous solution can be as long as 40 minutes. Most other enols are considerably more reactive than this, and many are too short-lived to be studied by these techniques. We consequently developed other methods which avoid mixing by generating the enol directly in the reaction medium. All of these faster methods use photochemical reactions and flash photolytic techniques.

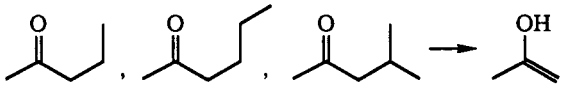
The first investigation of an enol by flash photolysis was performed by Wirz at the University of Basel and was based upon Norrish type II photoelimination of butyrophenone, eq. 2 (ref. 8). Irradiation of butyrophenone induces



(2)

hydrogen transfer from the γ-position to the carbonyl oxygen atom, and the diradical thus produced then breaks down

giving acetophenone enol plus ethylene. Working in collaboration with Wirz, we extended this method to aliphatic systems and produced the enol of acetone from the three ketones shown in eq. 3 (ref. 9). We also generated this enol



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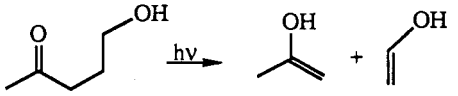
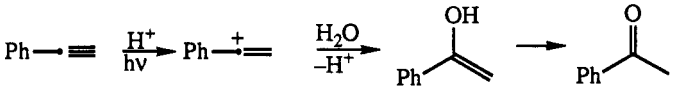


(4)

from the hydroxyketone shown in eq. 4 (ref. 10). The latter reaction gives the enol of acetaldehyde as well, but that enol is two orders of magnitude less reactive than the enol of acetone, and we were consequently able to make accurate measurements of the rates of ketonization of both enols in a single experiment.

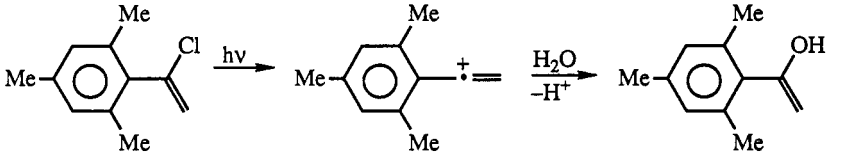
We have also made enols by the photohydration of acetylenes, eq. 5 (ref. 11). This reaction occurs thermally as well,



(5)

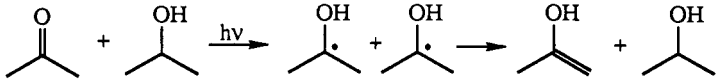
but, at the high acidities required to effect thermal protonation of the triple bond, ketonization of the enol is very rapid and this stage of the process is therefore a fast invisible reaction after the rate-determining step (ref. 12). Photoexcitation, however, increases the reactivity of acetylenes enormously (ref. 13), and in a flash photolysis experiment formation of the enol becomes more rapid than its ketonization; the enol may consequently be observed and studied.

Vinyl cations such as that shown in eq. 5 may also be produced by photosolvolysis of vinyl halides (ref. 14), and we have used this reaction to generate and study enols as well, eq. 6 (ref. 11c).



(6)

We have also prepared enols by photooxidation-reduction of alcohol-carbonyl compound pairs. When an aldehyde or ketone with no γ-hydrogen atom, *e.g.* acetone, is irradiated, Norrish type II photoelimination cannot take place. However, in the presence of a hydrogen atom donor such as isopropyl alcohol, intermolecular hydrogen atom transfer can occur, eq. 7; this produces two ketyl radicals which then disproportionate giving an enol and regenerating the alcohol. We have used this process to produce enol isomers of a number of aliphatic aldehydes and ketones (ref. 15)



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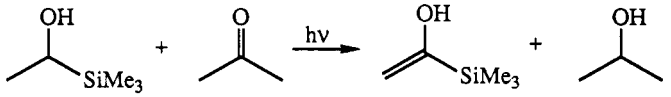
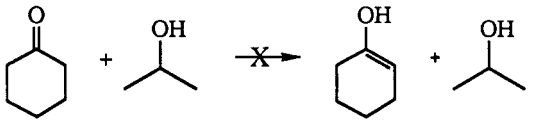
as well as the enol of acetyltrimethylsilane, eq. 8 (ref. 16).



(8)

We did not succeed in making the enol of cyclohexanone by this method when we used cyclohexanone and isopropyl alcohol as reactants, eq. 9, (although the other combination, cyclohexanol and acetone, did give cyclohexanone enol);



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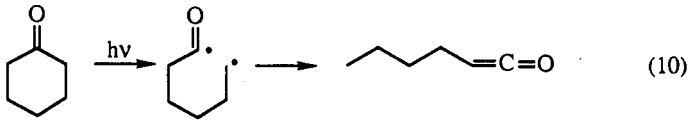
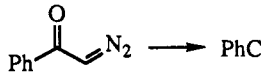
α-cleavage occurred instead, followed by intramolecular disproportionation to give *n*-butylketene eq. 10 (ref. 17). This took us into the chemistry of ketenes, which are interesting reactive species in their own right.

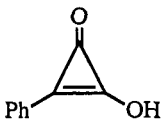


(10)

We have also made ketenes by the photo-Wolff reaction, eq. 11 (ref. 17), as well as by decarbonylation of phenylhydroxycyclopropenone, eq. 12 (ref. 18). The latter process first produces an ynol, the triple bond analog of



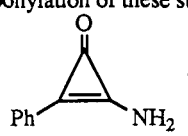
$$PhCH{=}C{=}O + N_2 \qquad (11)$$



$$\xrightarrow{h\nu} PhC{\equiv}COH \longrightarrow PhCH{=}C{=}O + CO \qquad (12)$$

an enol. Ynols are much more reactive than enols, and, although present in interstellar space, they were observed on earth only in the gas phase (ref. 19) and in an argon matrix (ref. 20) before our study of them in aqueous solution (ref. 18). Ynols are remarkable substances. They have proved to be very acidic: the pK_a of phenylynol (PhC≡COH), for example, is less than 2.8, which makes it stronger than most carboxylic acids and at least 7 pK units more acidic than its double-bond analog, the enol of phenylacetaldehyde (ref. 7b).

We have performed flash photolysis on amino analogs of phenylhydroxycyclopropenone as well (ref. 21). Decarbonylation of these substances gives ynamines, eq. 13, which also display the remarkable acid-strengthening



$$\xrightarrow{h\nu} PhC{\equiv}CNH_2 \rightarrow PhCH{=}C{=}NH + CO \qquad (13)$$

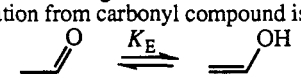
effect of carbon-carbon triple bonds: we have been able to set an upper limit of $pK_a \leq 18$ for phenylynamine ionizing as an acid, eq. 14, which makes it at least 17 pK units more acidic than ammonia (ref. 22). We have also observed

$$PhC{\equiv}CNH_2 \rightleftharpoons PhC{\equiv}CNH^- + H^+ \qquad (14)$$

that ynamines with at least one N-H bond isomerize to ketenimines, eq. 13, which are interesting and reactive nitrogen analogs of ketenes.

ENOL CHEMISTRY

One of the things that one would like to know about enols is the magnitude of equilibrium constants for their formation from carbonyl compound isomers, *i.e.* the values of of K_E for reactions such as that in eq. 15. The



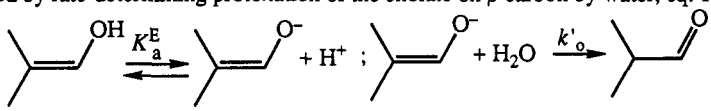
(15)

traditional way of determining such "enol contents", Kurt Meyer halogen titration (ref. 23), is based upon the fact that enols react with halogens whereas carbonyl compounds do not. This method works well when enol contents are high, but many substances other than enols react with halogens, and the method fails badly when enol contents are low. For example, the most recent determinations of K_E for cyclohexanone, made by three successive refinements of the Meyer method, differ by more than three orders of magnitude (ref. 24), and the lowest of these is ten times greater than the enol content that we determined for cyclohexanone recently by a method which is free of the difficulties attending halogen titration of substances at very low concentration (ref. 15).

Our method is based upon the fact that an equilibrium constant for a chemical reaction is equal to the ratio of its forward to reverse rate constants; thus, for the enolization reaction of eq. 15, $K_E = k_E/k_K$, where k_E is the specific rate of enolization of the carbonyl compound and k_K is that for ketonization of its enol.

Rates of enolization are easily measured, *e.g.* by halogen scavenging, and the literature abounds in values of k_E. Rates of ketonization have been more of a problem, but they may now be determined by generating the enol at greater than equilibrium concentration, using one of the methods we have devised, and then monitoring its conversion to the keto isomer. Such evaluations of K_E may be made under several sets of expeimental conditions, *e.g.* in acidic or basic solutions, for, as Figure 1 shows, ketonization, and therefore enolization as well, is catalyzed by both acids and bases. The equilibrium constant, of course, must remain invariant under changing catalyst conditions, and comparison of values obtained with different catalysts then affords a check on the reliability of the results.

Ketonization rate measurements made in basic solution also provide additional information about enol chemistry. The base catalysis seen in Figure 1 is produced by equilibrium ionization of the enol to the much more reactive enolate ion, followed by rate-determining protonation of the enolate on β-carbon by water, eq. 16; this makes the rate of the overall



(16)

process inversely proportional to $[H^+]$ and directly proportional to $[HO^-]$. Eventually, however, at sufficiently high values of pC_{H^+}, the prior equilibrium shifts over to the side of enolate ion, and the advantage of converting a less reactive to a more reactive species is lost; hydroxide-ion catalysis then becomes saturated. Standard treatment of rate

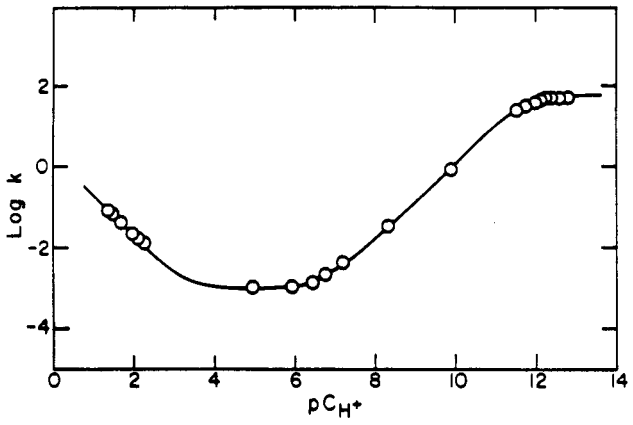
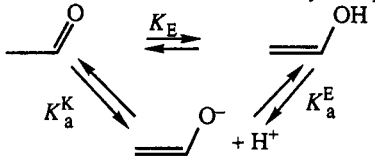


Figure 1.
Rate profile for the ketonization of isobutyraldehyde enol catalyzed by solvent-derived species in aqueous solution at 25°C; $pC_{H^+} = -\log[H^+]$.

data obtained in the region of change from catalysis to no catalysis gives both the rate constant for the rate-determining step and the equilibrium constant for the prior ionization step. Acid ionization constants of enols may therefore be determined in this way, and this may be done despite the fact that some enols are very short-lived species.

Keto-enol equilibrium constants and acid ionization constants of enols form two legs of a thermodynamic cycle whose third member is ionization of the carbonyl compound as a carbon acid, eq. 17. Acidity constants of carbonyl



(17)

compounds may therefore be calculated from the other two constants: $K_a^K = K_E K_a^E$.

Some carbonyl compounds are sufficiently acidic to allow determination of K_a^K directly in dilute aqueous solution. The thermodynamic cycle of eq. 16 then provides a check on the reliability of all of the data, for the product of equilibrium constants around the cycle must be unity. Diphenylacetaldehyde is one such case: we were, in fact, able to determine each of the three equilibrium constants for this substrate by two independent methods, and the product of these did prove to be unity within the combined experimental uncertainty of all of the results (±10%) (ref. 5b).

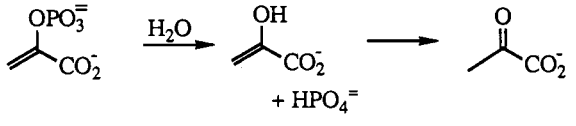
A sample of the enol chemistry that we have elucidated by these methods is given in Table 1. The enol contents which we obtained are uniformly smaller, often by several orders of magnitude, than those determined by halogen titration. Our results, on the other hand, are generally consistent with values that have been estimated by a variety of recent approximate methods (ref. 25); this shows that the assumptions upon which these approximate methods are based are essentially correct.

It may be seen from the data of Table 1 that the enol content of ketones is generally lower than that of aldehydes. This difference may be understood in terms of stabilization of the keto isomer by electron donation to its positively charged carbonyl carbon atom: ketones have two alkyl or aromatic groups to provide such stabilization whereas aldehydes have only one. Alkyl or phenyl substitution one carbon atom away from the carbonyl group, on the other hand, raises enol contents, this time by lowering the energy of the enol isomer through the well-known double-bond stabilizing effect of these groups (ref. 26). There is also an interesting ring-size effect which raises the enol content of cyclohexanone over that of analogous acyclic ketones.

The data of Table 1 show further that the acid strength of enols is comparable to that of phenols, and that there is not as much variation in enol acidity constants (pK_a^E) as there is in the acidity constants of aldehydes and ketones ionizing as carbon acids (pK_a^K). Changes in pK_a^K, moreover, parallel those in pK_E, and there is in fact a good linear correlation between these two quantities (ref. 27).

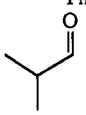
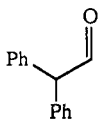
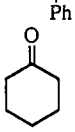
PYRUVIC ACID ENOL

Unfavorable biological reactions are often driven by being coupled to favorable transformations of high energy molecules, and one of the most energy-rich of such substances is phosphoenolpyruvate, the phosphate ester of the enol of pyruvate ion. The two-stage hydrolysis of this substance to pyruvate and hydrogen phosphate ions, eq. 18, is



(18)

TABLE 1. Keto-Enol Equilibrium Constants and Acid Ionization Constants of the Enol and Keto Forms for Some Simple Aldehydes and Ketones[a]

Substrate	pK_E	pK_a^E	pK_a^K
	6.23	10.50	16.73
	8.33	10.94	19.27
	7.96	10.34	18.31
	3.86	11.63	15.49
	0.98	9.40	10.42
	6.39	11.70	18.09

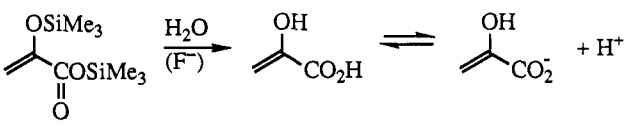
[a] In aqueous solution at 25°C, ionic strength = 0.10 *M*; constants are concentration quotients appropriate to this ionic strength.

TABLE 2. Keto-Enol Equilibrium Constants for the Pyruvic Acid System in Aqueous Solution at 25°C

Method	pK_E
$k^E_{H^+}/k^K_{H^+}$	3.13±0.03
k^E_{uc}/k^K_{uc}	3.20±0.03
Burst, $pC_{H^+} \approx 1.1$-1.2	3.16±0.02
$k^E_{HO^-}/k^K_{HO^-}$	5.12±0.01
Burst, $pC_{H^+} = 6.1$	4.97±0.10

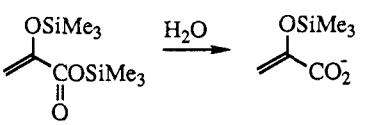
exoergic by $\Delta G° = -14.6$ kcal mol^{-1} (ref. 28). This is nearly twice the free energy liberated in the conversion of ATP to ADP, the main energy-producing reaction in biological systems; the reverse of the latter process, the unfavorable conversion of ADP to ATP, is in fact driven by the transformation of phosphoenolpyruvate to pyruvate.

Some of the energy released by the hydrolysis of phosphoenolpyruvate to pyruvate must be contributed by the second stage of this process, ketonization of enolpyruvate. In order to determine just how much that is, we have examined this keto-enol system (ref. 29).
We generated the enol from the corresponding *bis*-trimethylsilyl derivative, eq. 19 (ref. 30). This substance reacts



(19)

rapidly with water to give a transient species with strong absorbance in the region of the band, $\lambda_{max} = 225$ nm, reported for enol pyruvate (ref. 31). However, the silyl enol ether produced by hydrolysis of only the more labile acyloxysilyl group of the substrate, eq. 20, should absorb in the same region, and the transient observed could



(20)

therefore be this silyl ether. We were able to rule out that possibility from the effect of fluoride ion on the rate of decay of this transient: the hydrolysis of silyl ethers is catalyzed strongly by fluoride (ref. 2b,32), but addition of this ion to the reaction mixture had no effect on the lifetime of the transient. This indicates that the transient observed is not the silyl ether but the enol of pyruvic acid.

Decay of this enol is catalyzed by acids and bases, as expected, and its rate profile is typical of enol ketonization. Measurements in perchloric acid solutions give the ketonization rate coefficient $k^K_{H^+} = (6.5\pm0.4)\times10^{-3}\ M^{-1}\ s^{-1}$ for the hydronium-ion-catalyzed process and $k^K_{uc} = (1.00\pm0.04)\times10^{-2}\ s^{-1}$ for the "uncatalyzed" reaction (ref. 11b), and rate measurements in sodium hydroxide solutions provide the enol acidity constant $pK^E_a = 11.55\pm0.03$ and the specific rate of ketonization catalyzed by hydroxide ion $k^K_{HO^-} = (6.1\pm0.2)\times10^4\ M^{-1}\ s^{-1}$.

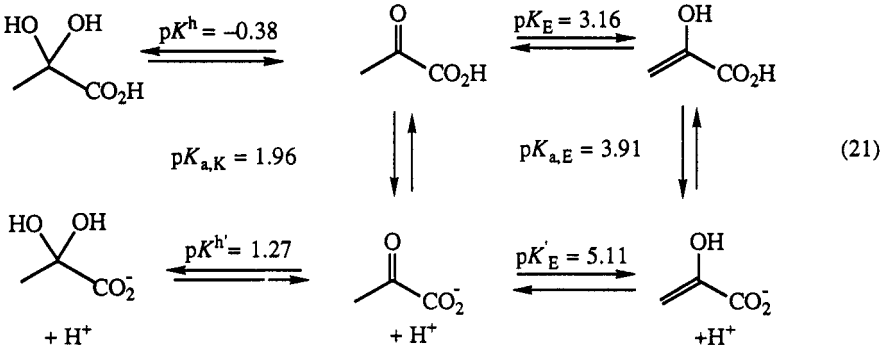
Rates of enolization of pyruvic acid were also determined, by iodine scavenging, and combination of these with the ketonization rate constants gives the keto-enol equilibrium constants listed in Table 2.

In these enolization experiments, rapid initial bursts of iodine consumption were observed preceeding the much slower uptake of iodine due to the enolization reaction. These bursts were attributed to reaction of iodine with enol present at equilibrium, and their magnitude was used, as in the Meyer halogen titration, to provide additional estimates of the keto-enol equilibrium constants. These are also listed in Table 2.

It may be seen that the statistical uncertainty associated with values of K_E measured by the burst method is significantly greater at high pC_{H^+}, where K_E is small, than at low pC_{H^+}, where it is considerably larger. Agreement between the results obtained by the burst method and by the ratio of enolization to ketonization rate constants is on the whole quite good.

The difference in enol content between low and high pC_{H^+} is an interesting consequence of the state of ionization of the system's carboxylic acid group. At low pC_{H^+} this group is of course present in the unionized carboxylic acid form, which is electron withdrawing and therefore exerts a destabilizing effect on the keto isomer through interaction with the positively charged carbon atom of its carbonyl group. At high pC_{H^+}, on the other hand, the carboxylic acid group is ionized and the negative charge it now has offsets the electron-withdrawing effect; destabilization of the keto form is therefore reduced and the enol content goes down.

Best values of the results obtained are displayed in the scheme of eq. 21. The acidity constant of pyruvic acid given



(21)

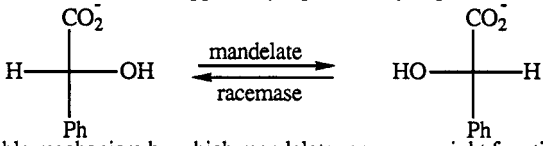
there, $pK_{a,K}$, is the conventional value, pK_a = 2.49 (ref. 33), corrected for hydration of the keto groups of the carboxylic acid and carboxylate forms (ref. 34); the other constants are also corrected for hydration as required.

Our keto-enol equilibrium constant for the carboxylate form, pK'_E = 5.11, agrees well with an estimate, pK'_E = 5.1, made previously (ref. 35) from rates of ketonization of enol produced enzymatically in acetic acid buffers and rates of enolization measured by iodine scavenging in similar solutions (ref. 36), and it is also consistent with pK_E = 5.4 determined by Meyer iodometric titration (ref. 37). The ionization constant of the carboxylic acid group of the enol, $pK_{a,E}$ = 3.91, which may be calculated from our results, is also in agreement with an estimate of this constant, $pK_{a,E}$ = 3.7, made using a free energy relationship (ref. 35).

Both pyruvic acid and its enol will exist in carboxylate forms at physiological p*H*, and the keto-enol equilibrium constant applicable to these forms is the quantity of interest in connection with the free energy of hydrolysis of phosphoenolpyruvate. The result obtained here, pK'_E = 5.11, corresponds to ΔG = -7.0 kcal mol^{-1}. This is 48% of the overall free energy of hydrolysis of phosphoenolpyruvate to pyruvate (eq. 18). Ketonization of enol pyruvate thus supplies neary half of the free energy liberated in the hydrolysis of phosphoenolpyruvate, and nearly half of the energy contained in this high energy substance is therefore due to the presence of a masked enol group.

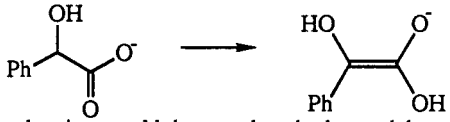
MANDELIC ACID ENOL

Enzymes are chiral catalysts that generally bind and process enantiomeric substrates with different efficiencies. There is thus unusual interest in enzymes such as mandelate racemase which converts either enantiomer of mandelate ion into the other isomer with apparently equal facility, eq. 22 (ref. 38).



(22)

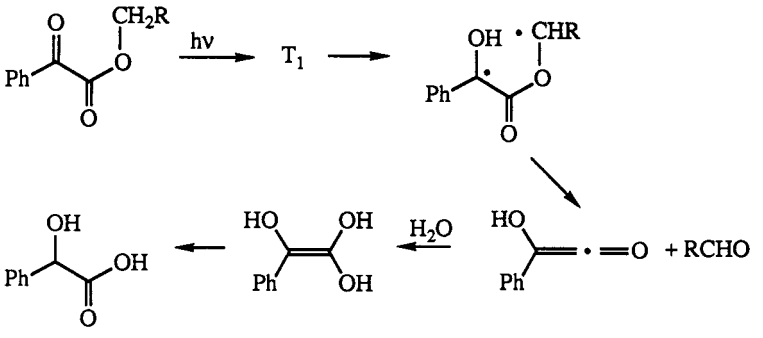
A possible mechanism by which mandelate racemase might function consists of enzymatically promoted enolization of mandelate ion, eq. 23, followed by release of the enolate product from the enzyme; reketonization of the no longer



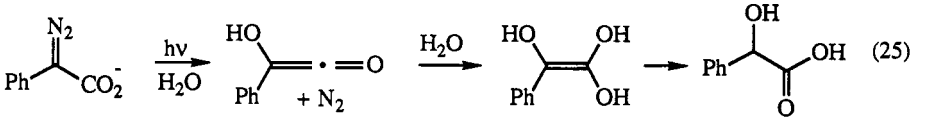
(23)

chiral enolate ion would then produce both mandelate enantiomers. Our study of the mandelic acid keto-enol system (ref. 39) has provided information bearing on this matter.

We prepared the enol of mandelic acid by two different flash photolytic processes, Norrish type II photoelimination of benzoylformate esters, eq. 24 R = H or *i*-Pr, and photo-Wolff reaction of phenyldiazoacetate, eq. 25. The Norrish



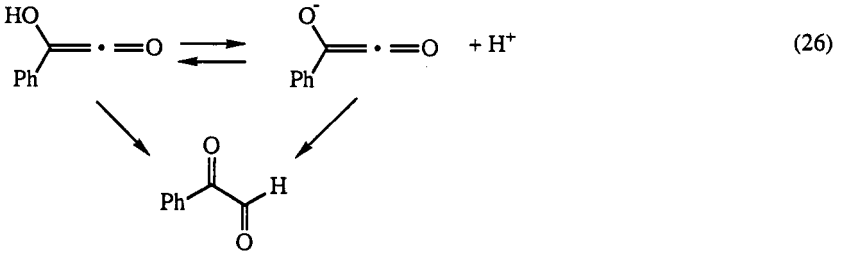
(24)



(25)

reaction produced three transient species; the photo-Wolff process, on the other hand, produced two, whose behavior was identical with that of the last two transients of the Norrish reaction.

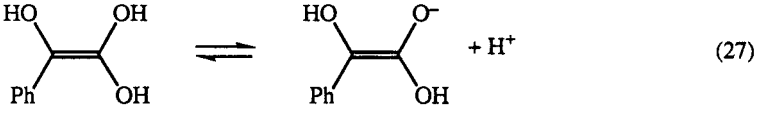
We identified the first transient of the Norrish reaction as the triplet excited state from its uv spectrum and lifetime (ref. 40), as well as its behavior toward acids, bases, and oxygen. The next transient is a bifunctional molecule which can, in principle, react either as a ketene as shown in eqs. 24 and 25, or as an enol, eq. 26. We could exclude the latter



(26)

possibility by the solvent isotope effect on the rate of decay of this transient, the form of acid-base catalysis, and the absence of phenylglyoxal, the expected product of the enol reaction (eq. 26), from the reaction product. This established decay of this transient as a ketene hydration reaction and indicated that the last transient was the enetriol enol of mandelic acid.

Decay of this enol gave a rate profile, Figure 2, which is typical of enol ketonization reactions. In addition to catalysis by hydronium and hydroxide ions, it showed strong catalysis by buffer species, as expected, and gave solvent isotope effects characteristic of ketonization reactions. The rate profile inflection point leading to saturation of hydroxide ion catalysis gives $pK_a^E = 6.62$ for acid ionization of the enol, eq. 27 (Note a), in good agreement with $pK_a^E = 6.45$



(27)

determined for the enol of the methyl ester of mandelic acid (ref. 41).

We measured rates of enolization of mandelic acid by monitoring acid-catalyzed racemization of the optically active substrate. This proved to be a very slow reaction, and high temperatures (138-155°C) and concentrated acids (1.1-4.5 *M* HCl) were needed to get rates that could be measured in a reasonable length of time. Rates of reaction in these concentrated solutions were proportional to acid molarity, as expected for an enolization reaction (ref. 42), only up to [HCl] = 2 *M*; above this concentration, another process with a steeper acidity dependence (probably nucleophilic displacement of the hydroxyl group of mandelic acid by a water molecule), began to contribute to the rate of

Note a: Ionization probably occurs preferentially from the hydroxyl group trans to phenyl, as written, for the trans enol of phenylacetaldehyde is a stronger acid than the cis enol (ref. 7b); ionization of the hydroxyl group geminal to phenyl can be ruled out because this would produce phenylglyoxal, which is not observed as a reaction product.

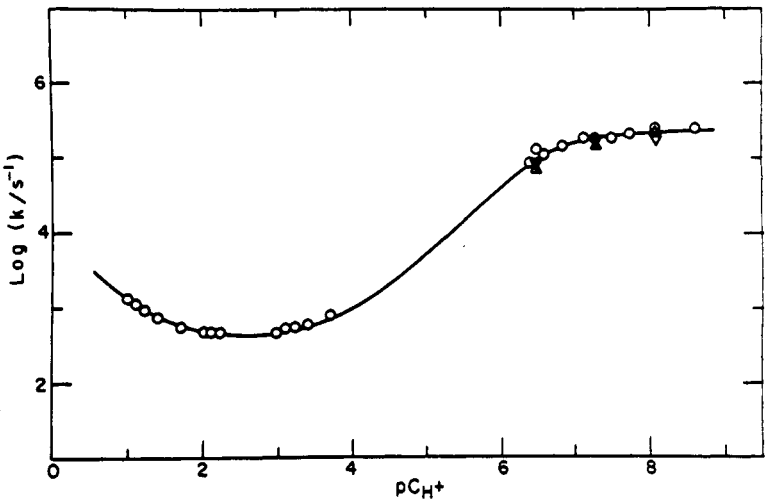


Figure 2. Rate profile for the ketonization of mandelic acid enol in aqueous solution at 25°C O: enol generated from methyl benzoylformate, Δ: enol generated from isopropyl benzoylformate, ∇: enol generated from phenyldiazoacetate.

racemization. Extrapolation to 25°C of data from the region where racemization was proportional to molarity gave the catalytic coefficient $k^{E}_{H^+} = 4\times10^{-12}\ M^{-1}\ s^{-1}$, and combination of that with the rate constant for ketonization provides $pK_E = 15.4$. This equilibrium constant plus the acidity constant of the enol, $pK^{E}_{a} = 6.62$, gives $pK^{K}_{a} = 22.0$ as the acidity constant of mandelic acid ionizing as a carbon acid.

These three equilibrium constants, pK_E, pK^{E}_{a}, and pK^{K}_{a}, are, to the best of our knowledge, the first ever measured for a simple carboxylic acid in aqueous solution (ref. 43). They show the enol content to be very low - orders of magnitude less than that of simple aldehydes and ketones - as expected from stabilization of the keto tautomer by interaction of the carbonyl and hydroxyl parts of the carboxylic acid group.

The bearing of our results on the racemization of mandelate ion catalyzed by mandelate racemase is illustrated in Figure 3. At physiological pH (= 7.00), mandelic acid enol lies above mandelate ion by ΔG = 25.9 kcal mol^{-1}, and the enolate ion lies above mandelate by 25.4 kcal mol^{-1}. The transition state for the enzyme catalyzed reaction, on the other hand, is much lower: the free energy of activation based upon k_{cat}/K_m, with k_{cat} = 1070 s^{-1} and K_m = 0.63 mM (ref. 38a) is only $\Delta G^{\neq}$ = 9.0 kcal mol^{-1}. Neither free enol nor free enolate ion can therefore be produced by the enzymatic reaction, and enzymatic enolization followed by release of enol or enolate ion plus ketonization off the enzyme cannot be the mechanism of the enzyme catalyzed reaction.

Enolization followed by ketonization, both within the enzyme active site, is, of course, still a possibility. In such a mechanism the enzyme would have to stabilize the enol and/or enolate ion by at least (25.4-25.9) - 9.0 = 16.4-16.9 kcal mol^{-1}; such stabilization might be supplied by coordination with the divalent metal cofactor which this enzymatic reaction requires.

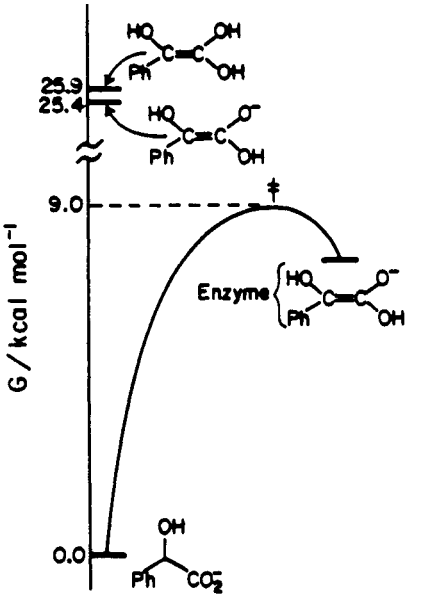


Figure 3. Energetic relationships in the mandelic acid keto-enol system at physiologial pH.

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