Electronic Factors Influencing the Decarboxylation of β-Keto Acids. A Model Enzyme Study

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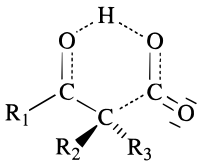
A theoretical study of the mechanism of decarboxylation of β-keto acids is described. A cyclic transition structure was found with essentially complete proton transfer from the carboxylic acid to the β-carbonyl group. The activation barrier for decarboxylation of formylacetic acid is predicted to be 28.6 kcal/mol (MP4SDTQ/6-31G*//MP2/6-31G*) while loss of CO_2 from its anion exhibits a barrier of only 20.6 kcal/mol (MP4SDTQ/6-31+G*//MP2/6-31+G*). Barrier heights of decarboxylation of malonic acid and α,α-dimethylacetoacetic acid are predicted to be 33.2 and 26.7 kcal/mol, respectively. Model enzyme studies using a thio methyl ester of malonate anion suggests that the role of malonyl-CoA is to afford a polarizable sulfur atom to stabilize the developing enolate anion in the transition structure for decarboxylation. Adjacent positively charged ammonium ions are also observed to stabilize the loss of CO_2 from a carboxylate anion by through-bond Coulombic stabilization of the transition structure.

Introduction

Decarboxylation is a key step for the biosynthesis of terpenoids, steroids, and neurotransmitter amino compounds. β-Keto acid decarboxylation is a known reaction in organic chemistry[1] as well as in biological systems.[2] Early mechanistic studies on the decarboxylation of β-keto acids have served two fundamental purposes. First, the loss of CO_2 is a key step in the malonic ester synthesis that is employed as a two-carbon extension procedure.[3] Second, decarboxylations of a variety of β-keto acid systems have been used as models for enzymatic reactions.[4,5] It has generally been accepted that the mechanism for the loss of CO_2 from β-keto acids involves a unimolecular decomposition that proceeds through a cyclic transition state (TS) resembling **1**.



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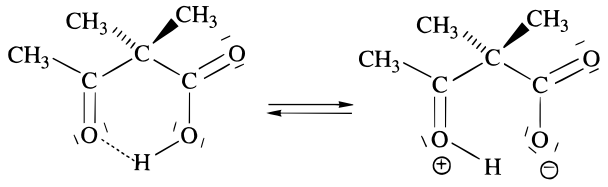
However, the position of the migrating hydrogen and the extent of carbon−carbon bond cleavage in the transition state remains an unresolved issue. Part of the controversy stems from earlier hydrogen isotope effect measurements that varied from 0.8 to 2.8 for k_H/k_D on the thermal decomposition of substituted benzoylacetic acids.[6] These data measured in benzene solvent are consistent with hydrogen transfer involvement in some systems but not in others. Substituent effects for these aryl-substituted systems exhibit a ρ of 0.03 in water and −1.0 in benzene solvent, suggesting a nonpolar TS in water but significant charge separation in benzene. However, it was observed later that enolization of benzoylacetic acid is extensive and that this contributed to the conflicting results since both the substituent and kinetic isotope effects were not confined strictly to decarboxylation.[1] The use of α,α-dimethylbenzoylacetic acid, a nonenolizable substrate, obviated this complicating side reaction. Relatively low isotope effects (k_H/k_D ∼1.3) for a series of aryl-substituted acids measured in water led to the conclusion that the hydrogen is not undergoing migration in the reaction coordinate.[1]

Solvent effects have also been employed to probe the nature of the transition structure for decarboxylation. In a pioneering study, Westheimer and Jones[7] showed that the rate of decomposition of α,α-dimethylacetoacetic acid in protic solvent is independent of the dielectric constant of the solvent. Thus, it was concluded that the reaction could not take place by way of dipolar intermediate **2b** since the rate would be expected to decrease sharply as the dielectric constant of the medium was lowered. The



2a **2b**

same conclusion was reached by Swain,[6] who extended the method to other acids. The opposite conclusion had been reached by Pedersen on the basis of his earlier mechanistic studies.[8] A transition state resembling **2b** has also been invoked to explain volumes of activation for β-keto acid decarboxylation.[9] However, Hine[9] suggested that although **2b** should be less readily formed in solvents of low dielectric constant, it should be more reactive because of the destruction of charge in the TS.

[⊗] Abstract published in *Advance ACS Abstracts,* July 15, 1996.

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(6) Swain, C. G.; Bader, R. F. W.; Esteve, R. M., Jr.; Griffin, R. N. *J. Am. Chem. Soc.* **1961**, *83*, 1951.
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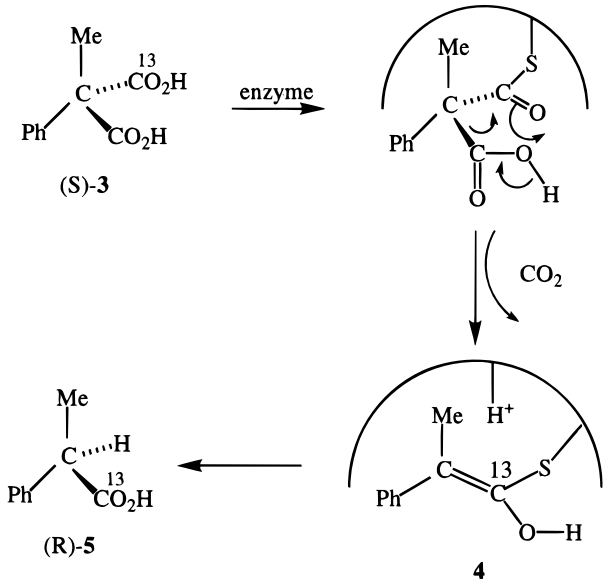
Scheme 1



Nonetheless, Bigley[10] preferred the uncharged structure **2a** on the basis of substituent effects. He did include the caveat that the O−H bond may be broken prior to the transition state but still result in an isotope effect if there is a rapid preequilibrium of the migrating hydrogen.

It has been suggested that β-keto acids have a built-in "electron sink" to stabilize the incipient carbanion formed on loss of CO_2 as an enolate ion.[2] The possibility of enolization attending C−C bond rupture is also generally thought to provide an example of electrophilic assistance to enzyme catalysis. Further extension of this concept suggests that protonation of the carbonyl group should in principle generate an even better "electron sink" and provide a neutral or free enol as the product. However, we feel that the low basicity of the carbonyl oxygen would not likely result in a kinetically meaningful concentration of fully protonated species under physiological conditions.

Decarboxylation of a β-keto acid is also a fundamental step in the enzyme-catalyzed biosynthesis of fatty acids. During enzymatic C−C bond condensation, malonyl-CoA serves as an activated two-carbon fragment that undergoes loss of CO_2 to provide an enolate anion for a subsequent acyl transfer. In a recent study of the stereochemistry of enzyme-catalyzed decarboxylation of α-methyl-α-phenylmalonic acid, Ohta[11] proposed the intermediacy of a thiol ester formed between a cysteine residue of the enzyme and the pro-*S*-carboxyl group of **3** (Scheme 1). The pro-*R* carboxyl group of **3** was eliminated to form (*R*)-**5** with inversion of configuration. One mechanistic possibility suggested involved enol **4** followed by enantioselective protonation on the *si*-face of the double bond. A concerted mechanism with retention of configuration has been proposed for methylmalonyl-CoA decarboxylation.[12]

In this report we describe transition structures for the decarboxylation of several β-keto acids. In each case the proton is essentially completely transferred to the carbonyl oxygen, affording a TS resembling dipolar ion **2b**. We provide a rationale for the unusual solvent effect where the rate of decarboxylation of zwitterion **2b** increases with decreasing dielectric constant of the solvent. We also suggest that an adjacent polarizable sulfur atom and a positively charged ammonium group can contribute to the rate acceleration of enzymatic decarboxylation. It is suggested that enzymatic decarboxylation proceeds by formation of its carboxylate anion or a zwitterionic intermediate.

Method of Calculation

Molecular orbital calculations were carried out using the Gaussian 94 program system[13a] utilizing gradient geometry optimization.[13b] All geometries were fully optimized using second-order Møller−Plesset perturbation theory (MP2/6-31G*). Reactants and transition states for the carboxylate anions were optimized at the MP2/6-31+G* level. Relevant energies and barrier heights were computed using fourth-order Møller−Plesset perturbation theory (frozen core, MP4SDTQ/6-31G*//MP2/6-31G*). Vibrational frequency calculations at the same level as optimization were used to characterize all stationary points as either minima (zero imaginary frequencies) or first-order saddle points (a single imaginary frequency). Thermodynamic quantities have been computed by making use of the harmonic oscillator−rigid rotor approximation and frequencies scaled by a factor of 0.8929.

Results and Discussion

Formylacetic Acid. We initiated our study with the simplest β-keto acid, formylacetic acid (**6**). In its ground state the carboxyl group is hydrogen bonded to the carbonyl (O_3-H_{10} = 1.829 Å, Figure 1a). The dihedral angle between the carbonyl group and the C−C bond that is broken in the decarboxylation process ($O_3-C_2-C_1-C_7$) is −28.8°, and the $C_2-C_1-C_7$ bond angle in ground state **6** is 117.6°. In the transition state, TS-**7**, the proton is essentially transferred at the carbonyl oxygen (O_3-H_{10} = 1.094 Å) and the C_1-C_7 bond distance has elongated from 1.532 to 1.803 Å. The $O_3-C_2-C_1-C_7$ dihedral angle has decreased to −50.4°, reflecting the developing planarity of the carbon−carbon double bond (C_2-C_1) of the enol product **8** (Figure 2). The dihedral angle between syn hydrogens (H_4 and H_6) in TS-**7** is 0.0°, and the C_1-C_2 bond distance has been shortened by 0.101 Å, indicating some double-bond character. The C_1-C_2 bond distance in enol **8** is 1.335 Å. The classical barrier for this decarboxylation process is predicted to be 28.5 kcal/mol and the $\Delta G^{\ddagger}_{298}$ is 26.9 kcal/mol ($\Delta H^{\ddagger}_{298}$ = 25.8 kcal/mol, $\Delta S^{\ddagger}_{298}$ = −3.9 cal/(mol K), MP2/6-31G*). The relatively small negative entropy of activation is consistent with a minimal change in molecular structure upon going from the intramolecular hydrogen-bonded ground state **6** to TS-**7**. The MP4SDTQ//MP2/6-31G* barrier for this unimolecular decomposition is 28.6 kcal/mol. Thus, the full fourth-order Møller−Plesset electron correlation correction has virtually no effect upon the activation barrier. The overall free energy predicted for this decarboxylation reaction is negative (ΔG_{298} = −8.8 kcal/mol, ΔH_{298} = 1.9 kcal/mol, ΔS_{298} = 36.0 cal/(mol K)), reflecting the rela-

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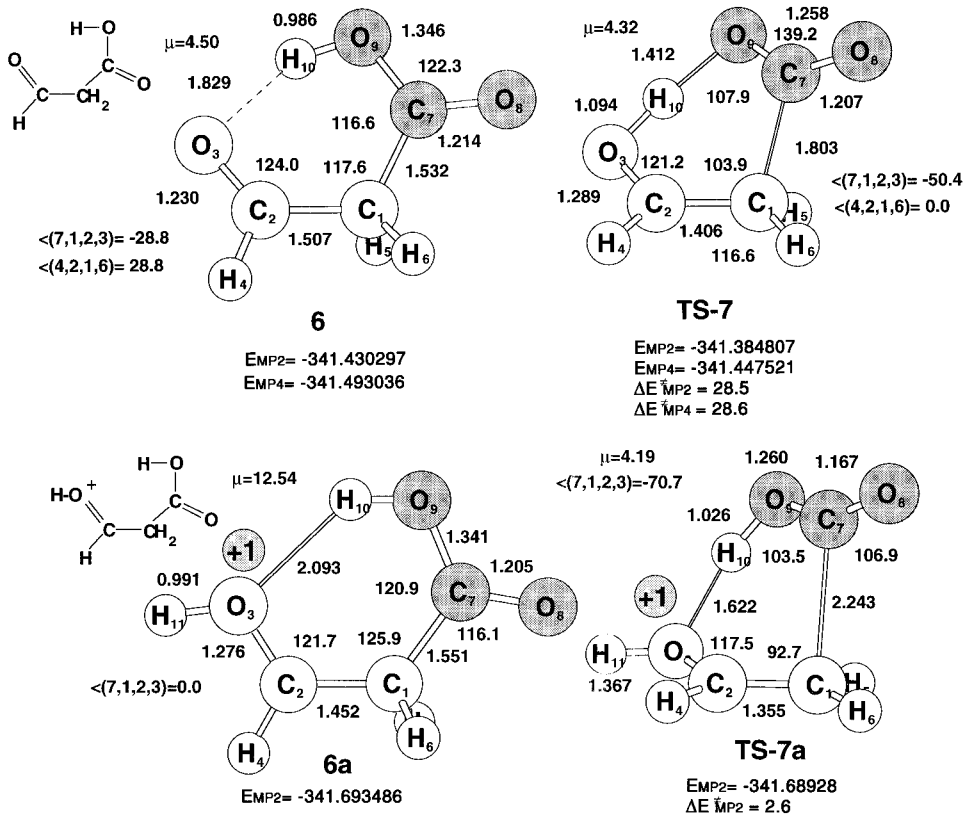

Figure 1. (a, top) Formylacetic acid (**6**) and its transition structure (TS-**7**) for decarboxylation. Geometries are fully optimized at MP2/6-31G*. Energies in Hartrees and activation barrier in kcal/mol. Distances in Å and angles in deg. The MP4 barrier is on MP2 geometries. Total dipole moments (μ) in D. The total energy for CO_2 at the MP2/6-31G* level is −188.107747. (b, bottom) Protonated formylacetic acid (**6a**) and its transition structure (TS-**7a**) for decarboxylation. Geometry of **6a** is optimized with the dihedral angle (7,1,2,3) constrained. TS-**7a** is fully optimized at MP2/6-31G*. Energies in Hartrees and activation barrier in kcal/mol. Distances in Å and angles in deg. Total dipole moments (μ) in D.

tively large increase in entropy as a consequence of the loss of CO_2. The driving force in this reaction may be only partially attributed to the formation of CO_2 since the energy difference between CO_2 and the CO_2 fragment in TS-**7** is 32.1 kcal/mol. The barrier height is largely due to the energetic requirements for the 1,5-hydrogen transfer ($O_9-H_{10}-O_3$) in TS-**7** since an optimized structure resembling **2b** with the O_3-H_{10} bond distance constrained at the same distance as that in TS-**7** (1.094 Å) is 24.9 kcal/mol above the energy of the ground state **6**. A modest decrease in dipole moment (−0.18 D) was noted upon going from ground state **6** to TS-**7**. Intramolecular proton transfer involving an intermediate resembling **2b** is not a viable possibility because **2b** does not exist as an energy minimum on the potential energy surface. It has been suggested that the highest probability for intramolecular proton transfer will occur when the cyclic TS formed can accommodate a linear arrangement of donor−proton−acceptor of appropriate length.[14] We found an $O_3-H_{10}-O_9$ bond angle of 151.0° for TS-**7**. For nonenzymatic decarboxylation of dimethylacetoacetic acid, Westheimer[7] has suggested a cyclic six-membered TS closely resembling the results reported herein when the free acid is the reactant.

A zwitterionic TS resembling dipolar structure **2b** appeared to be at odds with earlier predictions[6] that were based upon a small change in rate as the dielectric constant of the medium was decreased. Consequently we elected to examine the effect of a self-consistent

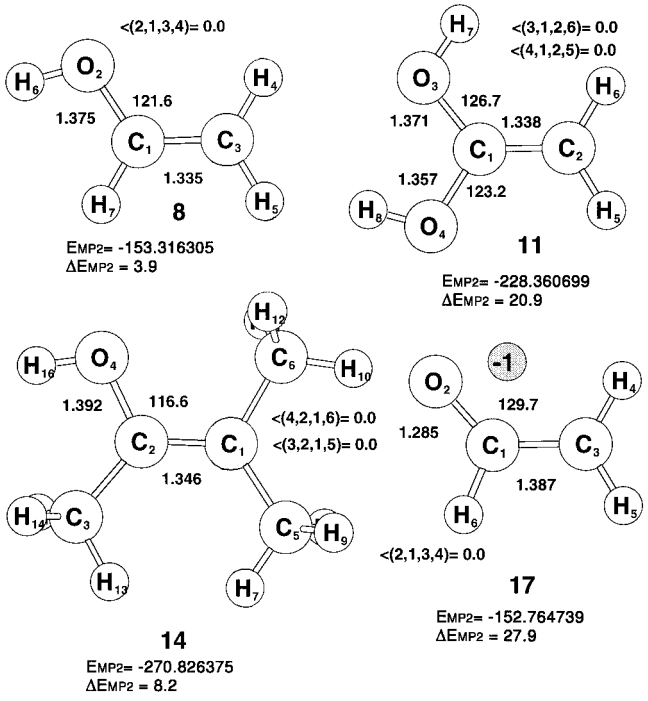

Figure 2. Products of decarboxylation of formylacetic acid **8**, malonic acid **11**, α,α-dimethylacetoacetic acid (**14**), and formylacetate anion **17**. Geometries fully optimized at MP2/6-31G* or MP2/6-31+G* for the anion **17**. Energies in Hartrees. Distances in Å and angles in deg. The total energy of CO_2 at the MP2/6-31+G* level is −188.117959. The ΔE_{MP2} for the overall decarboxylation process is given in kcal/mol.

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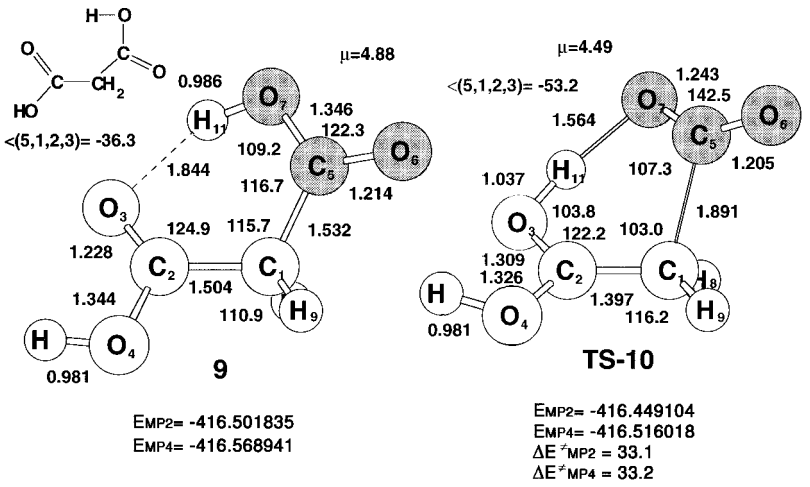

Figure 3. Malonic acid **9** and its transition structure (TS-**10**) for decarboxylation. Geometries fully optimized at MP2/6-31G*. Energies in Hartrees and activation barrier in kcal/mol. Distances in Å and angles in deg. The MP4 barrier is on MP2 geometries. Total dipole moments (μ) in D.

reaction field (SCRF) treatment of the activation barrier. Since many of the earlier mechanistic studies were carried out in water and water−alcohol mixtures, we used a dielectric constant (ϵ) of 76.7 (H_2O). Using the Tomasi method[13c] on the MP2/6-31G* geometry of TS-**7**, we observed a modest increase in the barrier height to 30.3 kcal/mol ($\Delta\Delta E^{\ddagger} = 1.8$ kcal/mol). Since the reaction rate of decarboxylation of malonic acid as a function of pH has been investigated,[15] we also examined the effect of full protonation on the reaction barrier. The protonated planar form of formylacetic acid (**6a**) does not exist on the potential energy surface as a minimum in the open form. In the gas phase it prefers to ring close to a protonated β-lactone. Consequently structure **6a** (Figure 1b) is a constrained minimum (one imaginary frequency). The barrier for decarboxylation computed from this minimum is only 2.6 kcal/mol. However, due to the low basicity of a typical carbonyl group, the concentration of protonated acid would be too low to significantly contribute to the reaction rate. This is consistent with the observation of only a modest increase in the rate of decarboxylation with added HCl.[15]

Malonic Acid. Mechanistic studies have also been reported for decarboxylation of several malonic acids.[15−18] The rate of loss of CO_2 from malonic acid in water at 80 °C increases with increasing acid concentration. It has also been shown that the monoanion exhibits a decarboxylation barrier of 28.5 kcal/mol.[15] The monoanion of phenylmalonic acid decomposes three−four times faster than the undissociated acid, and the overall rate increases with decreasing dielectric constant of the water−dioxane solvent mixture.[16] Since kinetic data are available for malonic acid decarboxylation, we extended this theoretical study to malonic acid and its derivatives. The decarboxylation of malonic acid (**9**, Figure 3) is endothermic overall ($\Delta G_{298} = 6.8$ kcal/mol, $\Delta H_{298} = 18.1$ kcal/mol, $\Delta S_{298} = 37.8$ cal/(mol K)) and has a slightly higher classical barrier height ($\Delta E^{\ddagger} = 33.1$ kcal/mol) than the loss of CO_2 from **6** ($\Delta G^{\ddagger}_{298} = 31.4$ kcal/mol, $\Delta H^{\ddagger}_{298} = 30.6$ kcal/mol, $\Delta S^{\ddagger}_{298} = -2.7$ cal/(mol K)). The SCRF barrier of 34.1 kcal/mol and $\Delta H^{\ddagger}_{298}$ of 31.6 kcal/mol ($\epsilon = 76.7$) are also slightly higher, consistent with the predictions above for β-keto acid **6** and in close agreement with experiment where the experimental activation energy is 30.8 kcal/mol.[15] The MP4SDTQ//MP2/6-31G* classical barrier height for decarboxylation of **9** is 33.2 kcal/mol. Most of the features of TS-**10** do not differ significantly from those discussed above for TS-**7**. It is important to point out that the developing double bond in TS-**10** remains highly twisted (dihedral $C_5-C_1-C_2-O_3 = -53.2°$) and the hybridization at C_1 is intermediate between sp^3 and sp^2 ($\Sigma_\theta = 346.8°$). The sum of the three angles about a developing sp^2 carbon ($\Sigma_\theta = 360°$) is an approximate indication of its deviation from sp^3 hybridization ($\Sigma_\theta = 328.4°$). The C_1-C_2 double bond in TS-**10** is about 78% developed ($R(C_1-C_2) = 1.338$ Å) relative to the double bond in enol **11** (Figure 2).

Effect of Alkyl Substituents. We next examined the effect of alkyl substituents on the geometry and energetics of decarboxylation. The ground state of α,α-dimethylacetoacetic acid (**12**, Figure 4) exists in a cyclic hydrogen-bonded configuration that closely resembles that in TS-**13** except that the acidic hydrogen is almost completely transferred in the transition state ($O_3-H_{10} = 1.066$ Å). This reaction is only slightly endothermic with $\Delta H_{298} = 5.7$ kcal/mol, ($\Delta G_{298} = -6.1$ kcal/mol, $\Delta S_{298} = 39.5$ cal/(mol K)) despite the potential stabilization of the double bond due to the alkyl substituents on enol **11**. The classical barrier ($\Delta E^{\ddagger}$) for this decarboxylative process is predicted to be 26.8 kcal/mol ($\Delta G^{\ddagger}_{298} = 24.7$ kcal/mol, $\Delta H^{\ddagger}_{298} = 24.2$ kcal/mol, $\Delta S^{\ddagger}_{298} = -1.6$ cal/(mol K)). The reduction in barrier height is only 1.7 kcal/mol relative to the parent β-keto acid **6**, and this is possibly a reflection of only partial development of double-bond character between C_1 and C_2. The C_1-C_2 bond distances in reactant **12**, TS-**13**, and enol product **14** (Figure 2) are 1.531, 1.419, and 1.346 Å, respectively. The developing double bond in TS-**13** is only about 60% developed and has not yet attained planarity based upon a $O_3-C_2-C_1-C_8$ dihedral angle of −48.9°. Although the syn methyl groups in TS-**13** are eclipsed (dihedral $C_7-C_2-C_1-C_9 = 0.7°$), the hybridization at C_1 is still intermediate between sp^3 and sp^2 ($\Sigma_\theta = 345.5°$). The disruption in overlap between C_1 and C_2 is largely due to the deviation of oxygen (O_3) from the plane of the developing double bond. It has been postulated that the rate of decarboxylation

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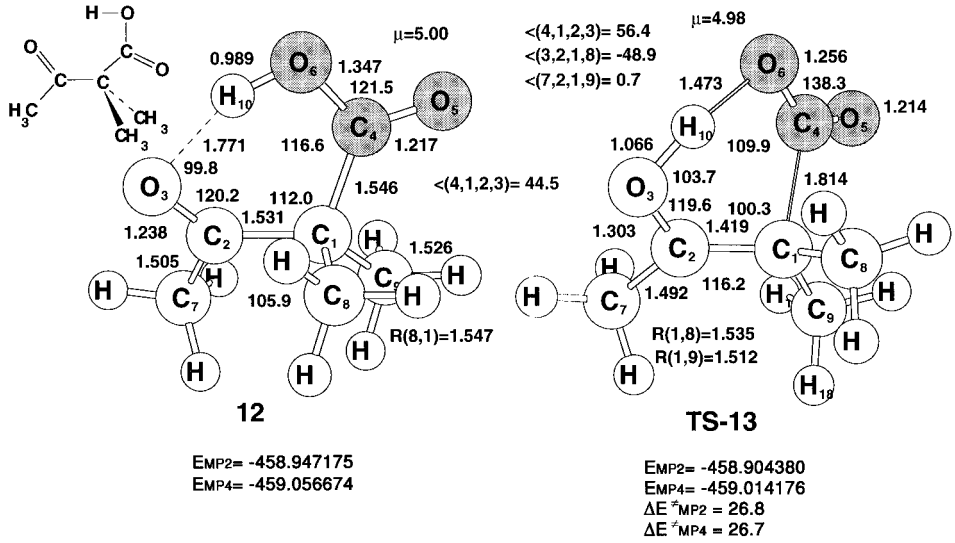

Figure 4. α,α-Dimethylacetoacetic acid (**12**) and its transition structure (TS-**13**) for decarboxylation. Geometries fully optimized at MP2/6-31G*. Energies in Hartrees and activation barrier in kcal/mol. Distances in Å and angles in deg. The MP4 barrier is on MP2 geometries. Total dipole moments (μ) in D.

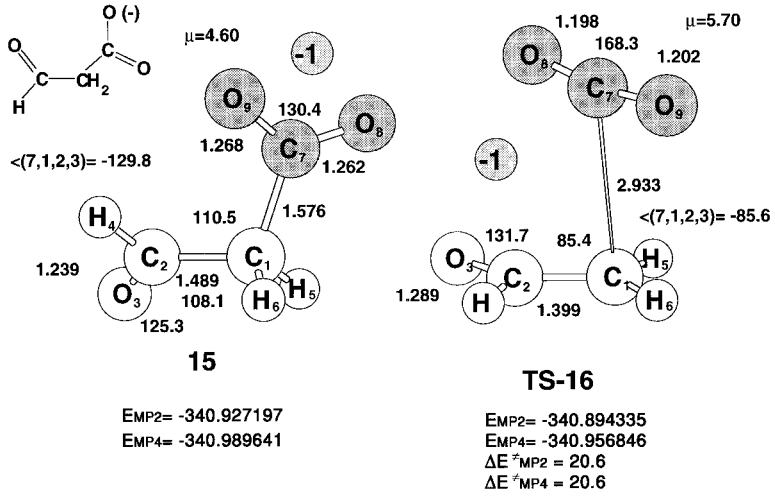

Figure 5. Formylacetate anion (**15**) and its transition structure (TS-**16**) for decarboxylation. Geometries fully optimized at MP2/6-31+G*. Energies in Hartrees and activation barrier in kcal/mol. Distances in Å and angles in deg. The MP4 barrier is on MP2 geometries. Total dipole moments (μ) in D.

is proportional to the cosine of the angle between the orbital of the incipient sp^3 carbanion and the p orbital of the carbonyl group.[19] The cyclic hydrogen-bonded TS is consistent with experiments that suggest that decarboxylation under typical synthetic conditions proceeds by way of the free acid.[6] Metal ion catalysis has also been observed for this decomposition.[4] The activation energy for decarboxylation of benzoylacetic acid in aqueous solution is 22.8 kcal/mol,[5] while we predict a $\Delta H^{\ddagger}_{298}$ = 24.2 kcal/mol for the comparable acetyl-substituted acetic acid. The experimental barrier for the benzoylacetate anion increased to 27.1 kcal/mol.[5] However, it is conceivable that enolization could have complicated the overall mechanistic picture and contributed to the barrier. As noted for the two related β-keto acids, the change in dipole moment upon going from ground to transition state is minimal, consistent with the trends in reaction rate as the dielectric constant of the solvent is decreased. Thus it appears that inclusion of alkyl groups does not significantly affect the overall potential energy surface for decarboxylation relative to the parent β-keto acid **6**.

In general the carboxylate anion of a β-keto acid should have a higher ground state energy than its corresponding acid and hence exhibit a lower barrier height for decarboxylation. Indeed, the anion of formylacetic acid (**15**, Figure 5) has a classical activation barrier of 20.6 kcal/mol[20] ($\Delta G^{\ddagger}_{298}$ = 17.2 kcal/mol, $\Delta H^{\ddagger}_{298}$ = 18.5 kcal/mol, $\Delta S^{\ddagger}_{298}$ = 4.2 cal/(mol K), MP2/6-31+G*) which is 7.9 kcal/mol lower than that predicted for the neutral β-keto acid **6**. The overall reaction is quite endothermic (ΔE = 27.9 kcal/mol, ΔG_{298} = 15.0 kcal/mol, ΔH_{298} = 25.3 kcal/mol, ΔS_{298} = 34.8 cal/(mol K)), possibly reflecting the electron delocalization of a carboxylate versus an enolate anion. The C_1-C_7 distance in TS-**16** is 2.933 Å, and the C_1-C_2 double bond of the enolate is almost completely formed (R = 1.399). The C_1-C_3 bond distance in the enolate product **17** is 1.387 Å (Figure 2). The reduced $\Delta G^{\ddagger}_{298}$ for this reaction (9.7 kcal/mol) relative to that for decarboxy-

(19) Ferris, J. P.; Miller, N. C. *J. Am. Chem. Soc.* **1966**, *88*, 3522.

(20) The activation barrier of this decarboxylation reaction has been recomputed both at the MP2/6-31G* and RHF/6-31+G* levels to assess the effect of diffuse functions in the basis set and the electronic correlation, respectively. Diffuse functions show minimal effect on the barrier, the latter being dimished by only 0.4 kcal/mol. The HF/6-31+G* barrier increased by 5.5 kcal/mol with respect to the MP2/6-31+G*.

lation of the neutral β-keto acid **6** is also a consequence of the enthalpy of formation of the second developing carbonyl π-bond. The CO_2 moiety in TS-**16** is almost completely formed since the energy of the CO_2 fragment in TS-**16** is only 1.9 kcal/mol higher than that in ground state CO_2. The C−O bond distances in TS-**16** are 1.198 and 1.202 Å while that in CO_2 is 1.181 Å. These data provide support for the underlying concept that enzymatic decarboxylation at physiological pH and temperature proceeds via the carboxylate anion hydrogen bonded to a counterion.

Electronic Factors. We next address electronic factors that influence the efficacy of the "electron sink" in these decarboxylation reactions.[2] It has been assumed generally that protonation of the carbonyl oxygen as in **2b** makes the keto group positively charged which in turn will increase the rate of decarboxylation. However, this can be misleading because the positive charge on the oxygen atom in **2b** is a formal charge that arises from a convention that is generally used for electron bookkeeping. The computed Mulliken or ChelpG charge on these atoms bears no resemblance to the formal charge, and as such formal charges should never be used to make mechanistic predictions. For example, the ChelpG[21] charge on the carbonyl carbon and oxygen of acetone and protonated acetone are 0.53, −0.54 and 0.60, −0.03, respectively. The calculated charges on the carbonyl group of formylacetic acid (**6**) are 0.507 and −0.449 for C_2 and O_3, respectively. In TS-**7** (Figure 1) these charges are 0.421 and −0.471. The calculated charge on the oxygen atom (O_3) that has a formal charge of +1 has actually become slightly more negative. The O_9-H_{10} bond distance in TS-**7** is much shorter than it would be in **2b** as a consequence of $C_2-C_1-C_7$ bond angle contraction in the TS. However, it is the charge in the TS that is pertinent to the rate and not those in **2b** since the latter does not exist as an energy minimum on this hypersurface. The alteration in charges and geometry result in a slight lowering of the dipole moment on going from **6** to TS-**7** (Figure 1).

The above arguments suggest that facile decarboxylation is not a consequence of a protonated carbonyl group in the TS for decarboxylation. Since the barrier for carboxylate anion decomposition (TS-**16**) is actually lower than that where the carbonyl oxygen is protonated (TS-**7**), we must search for another explanation for how enzymatic decarboxylation can occur readily at temperatures as low as 30 °C. Although the thiol ester functionality in coenzyme A can facilitate acyl transfer, the possibility exists that the polarizable sulfur atom can also help to stabilize the developing enolate anion in the TS for decarboxylation. In an effort to model the enzyme-bound enol **4**, we have examined the decomposition of the thiol ester of malonate carboxylate anion **18** (Figure 6). It is well-known that C−H bonds adjacent to one or more sulfur atoms results in a dramatic lowering of their pK_a as a result of carbanionic stability derived from the increased polarizability of this second-row atom.[22] Indeed, the barrier height for decarboxylation in TS-**19** is 4.5 kcal/mol lower than that for loss of CO_2 from the anion of formylacetic acid **16**. We suggest that this relatively modest reduction in activation energy is a consequence of an electronic factor associated with the

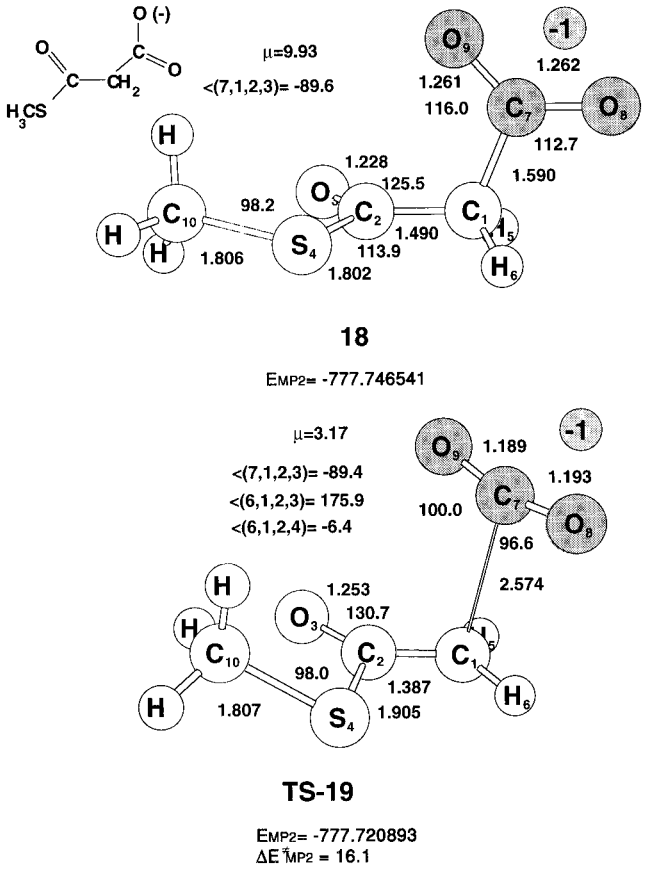

Figure 6. Thiomalonate methyl ester **18** and its transition structure (TS-**19**) for decarboxylation. Geometries fully optimized at MP2/6-31+G*. Energies in Hartrees and activation barrier in kcal/mol. Distances in Å and angles in deg. Total dipole moments (μ) in D.

ability of the sulfur atom to disperse negative charge. The decarboxylation process is attended by a dramatic decrease of the dipole moment computed with the HF wave function ($\Delta\mu = -6.76$ D), and the formation of CO_2 in the TS is nearly complete. The magnitude of the activation barrier ($\Delta E^{\ddagger} = 16.1$ kcal/mol, $\Delta G^{\ddagger}_{298} = 12.5$ kcal/mol, $\Delta H^{\ddagger}_{298} = 14.1$ kcal/mol, $\Delta S^{\ddagger}_{298} = 5.1$ cal/(mol K), MP2/6-31+G*) suggests the possibility that the overall decarboxylation process involving malonyl-CoA proceeds via the anion of the carboxylic acid. The sp^2 hybridization of C_1 is almost completely attained in TS-**19** ($\Sigma_\theta = 357.2°$). Enantioselective protonation of the enolate anion after the barrier has been crossed must proceed from the surface of the enzyme to the *si*-face of the double bond of the enolate anion of **4**.

To further test this hypothesis, we examined the influence of a modestly electronegative amino substituent on the rate of decomposition of formylacetic acid anion (Figure 7). We actually find a modest increase in the barrier height of 3.4 kcal/mol for TS-**21** ($\Delta E^{\ddagger} = 24.0$ kcal/mol, $\Delta G^{\ddagger}_{298} = 20.3$ kcal/mol, $\Delta H^{\ddagger}_{298} = 22.2$ kcal/mol, $\Delta S^{\ddagger}_{298} = 6.4$ cal/(mol K), MP2/6-31+G*) relative to that of its parent TS-**16**. This result would tend to rule out inductive stabilization due to electronegativity and may suggest an element of electron repulsion of the developing anion with the nitrogen lone pair of electrons.

We have previously suggested that adjacent positively charged ammonium ions can dramatically lower the barriers for oxygen atom transfer from 4α-flavin hydro-

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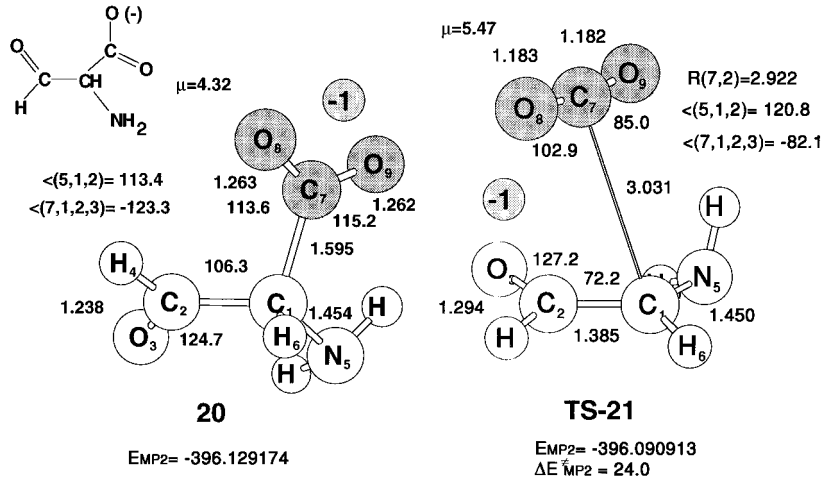

Figure 7. 2-Aminoformylacetate anion (**20**) and its transition structure (TS-**21**) for decarboxylation. Geometries fully optimized at MP2/6-31+G*. Energies in Hartrees and activation barrier in kcal/mol. Distances in Å and angles in deg. Total dipole moments (μ) in D.

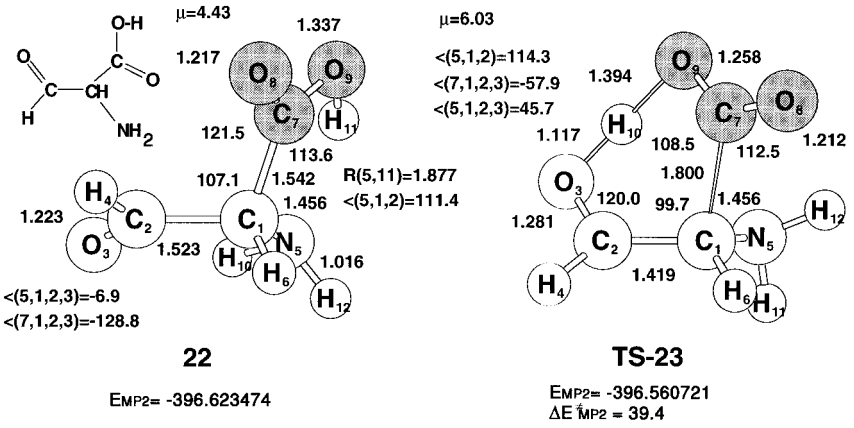

Figure 8. 2-Aminoformylacetic acid (**22**) and its transition structure (TS-**23**) for decarboxylation. Geometries fully optimized at MP2/6-31G*. Energies in Hartrees and activation barrier in kcal/mol. Distances in Å and angles in deg. Total dipole moments (μ) in D.

peroxides.[23] Positively charged imminium ions are postulated to influence the mechanism for pyruvamide and PLP-dependent enzymes.[24−26] Consequently we elected to examine the influence of a nitrogen atom bearing a formal positive charge on the barrier for loss of CO_2 (TS-**24**). We examined first the barrier for decarboxylation of a neutral α-amino-β-keto acid as a point of reference. As noted above, the loss of CO_2 is preceded by an intramolecular proton transfer that serves to minimize the charge separation in the TS. The proton transfer in α-amino-β-keto acid **22** can involve the carbonyl oxygen or the more basic nitrogen atom. The former pathway produces a neutral enol upon loss of CO_2 while the latter involves the formation of a zwitterionic α-amino acid along the reaction coordinate. Loss of CO_2 from neutral acid **22** is associated with an activation barrier of 39.4 kcal/mol ($\Delta G^{\ddagger}_{298} = 36.8$ kcal/mol, $\Delta H^{\ddagger}_{298} = 36.2$ kcal/mol, $\Delta S^{\ddagger}_{298} = -2.2$ cal/(mol K), MP2/6-31G*) if the necessary 1,5 proton transfer for decarboxylation occurs to the carbonyl oxygen (TS-**23**) (Figure 8). This decarboxylation process involves a substantial increase in dipole moment (1.60 D), and the barrier height is 10.9 kcal/mol higher than that for β-keto acid **6**. The increased barrier is due in part to the loss the strong hydrogen bond of the carboxylic acid to nitrogen in reagent **22** ($N_5-H_{11} = 1.877$ Å) while the hydrogen is transferred to the carbonyl oxygen ($O_3-H_{10} = 1.117$ Å) in TS-**23**.

Although it is well recognized that zwitterionic structures have higher energy than neutral species in the gas phase, the barrier height for decarboxylation of **22** is significantly reduced in TS-**24**, (Figure 9, $\Delta E^{\ddagger} = 19.0$ kcal/mol, $\Delta G^{\ddagger}_{298} = 18.3$ kcal/mol, $\Delta H^{\ddagger}_{298} = 17.5$ kcal/mol, $\Delta S^{\ddagger}_{298} = -2.9$ cal/(mol K), MP2/6-31G*) where the proton transfers to the more basic amino group, resulting in a fully developed ammonium substituent ($^{+}NH_3$ group charge of 0.49).

This raises the interesting question as to how the decrease in activation energy is partitioned between the Coulombic stabilization due to the positively charged ammonium substituent and the increase in ground state energy due to charge separation in the gas phase. Computing the barrier from the zwitterionic form **25** (Figure 10) gives a $\Delta E^{\ddagger}$ of only 5.9 kcal/mol. Since zwitterionic structure **25** is not a critical point on the gas phase surface, we had to constrain the N−H bond distances to prevent proton migration. This constrained structure, where the N−H bond distances are the same

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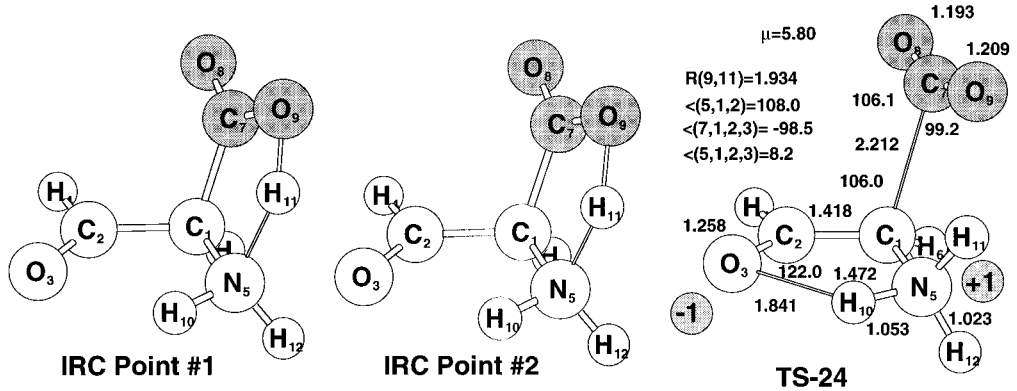

	R(1,7)	R(9,11)	R(5,11)	ΔE
IRC Point #1	1.587	1.144	1.400	7.4
IRC Point #2	1.594	1.241	1.307	9.9
TS	2.212	1.934	1.035	19.0

Figure 9. IRC on 2-aminoformylacetic acid decarboxylation reaction. Structures #1 and #2 correspond to two points on the IRC reaction path. Geometries are fully optimized at MP2/6-31G*. Energy in Hartrees and activation barrier in kcal/mol. Distances in Å and angles in deg. Total dipole moment (μ) in D.

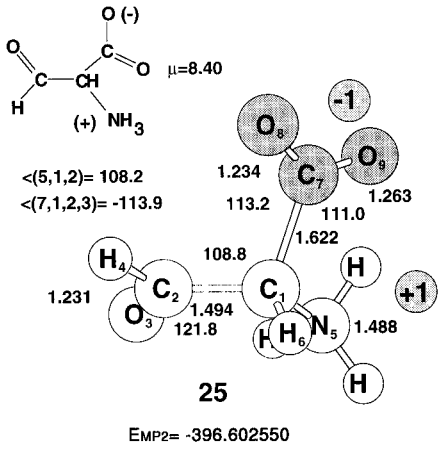

Figure 10. N-Protonated 2-aminoformylacetate **25**. H−N distances are constrained to 1.017 Å. All the other variables optimized at MP2/6-31G*. Energy in Hartrees. Distances in Å and angles in deg. Total dipole moment (μ) in D.

as those in TS-**24**, lies 13.1 kcal/mol above neutral α-amino-β-keto acid **22**. Thus the major portion of the barrier height relative to neutral **22** ($\Delta E^{\ddagger}$ = 19.0 kcal/mol) is a consequence of the charge separation in zwitterionic TS-**24**.

In order to better understand the different contributions to the reaction barrier for decarboxylation of **22**, we also performed an intrinsic reaction coordinate[27] (IRC) calculation connecting TS-**24** to the reactant (Figure 9). At the second point, which is intermediate along the reaction coordinate, the C_1-C_7 bond distance is only 8.4% elongated and the energy rise is 52% of the total barrier. The major geometric change related to this energy difference is the transfer of the carboxylic proton to the amino group. The barrier for proton transfer to the β-carbonyl group (TS-**23**) is 20.4 kcal/mol higher in energy than proton transfer to the α-amino substituent (TS-**24**). It is particularly significant that the proton transfer to N_5 is complete before the barrier is crossed in order to maximize the Coulombic stabilization in the TS. It is also emphasized that the positive charge on the nitrogen in **25** is a formal charge and that most of the positive charge will reside on the hydrogens. However, the net charge on the $^{+}NH_3$ fragment in **25** is positive (+0.51).

Conclusions. In summary, we have found a cyclic TS for decarboxylation of β-keto acids closely resembling TS-**1** that had been predicted earlier. However, in TS-**7** the proton has been completely transferred to the developing enol oxygen. The classical barrier ($\Delta E^{\ddagger}$ = 33.1 kcal/mol) for decarboxylation of malonic acid is slightly higher than that found for the simplest β-keto acid, formylacetic acid ($\Delta\Delta E^{\ddagger}$ = 4.6 kcal/mol). Loss of CO_2 from the anion of formylacetic acid resulted in a substantial lowering of the barrier height ($\Delta E^{\ddagger}$ = 20.6 kcal/mol). Both the thio ester functionality and positively charged ammonium ions can result in a lowering of the barrier height for decarboxylation. Such zwitterionic structures where the imminium ion bears a positive charge have been postulated to facilitate decarboxylation in PLP-dependent enzymes.[24−26] These data suggest that at physiological pH loss of CO_2 can be stabilized by through-bond Coulombic interactions.

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