spectra that are identical with those of the wild-type protein.[16-18] The addition of camphor to the mutant protein induces a low- to high-spin transition of the ferric center analogous to the native cytochrome P-450$_{cam}$. The dissociation constant for the substrate camphor to T252A was found to be 0.85 μM (25 °C), essentially equivalent to the wild-type value of 0.57 μM.[19,20] NADH consumption rates, and the production of 5-*exo*-hydroxycamphor as a sole product by the wild-type and mutant, also suggest that the integral protein conformation is conserved. In contrast, autoxidation of the ferrous, oxygenated species was drastically altered (Figure 1). The presence of substrate[23] and the structural features induced by threonine at position 252 stabilizes the one-electron-reduced, dioxygen-bound P-450 species (Figure 1). Similar activation energies for autoxidation suggest that camphor stabilizes the wild-type and the T252 mutant to the same extent and that variations in pre-exponential factors are primarily responsible for the 10-fold increase in autoxidation observed with T252A. Thus, as suggested by Poulos, T252 appears to be involved in stabilizing the oxygen complex.[10]

Although one-electron autoxidation is greatly enhanced, it cannot solely account for the fate of the NADH-derived reducing equivalents in the mutant protein, as the rate of NADH-coupled electron transfer into cytochrome P-450 is significantly faster than the autoxidation rate in both the wild-type and the mutant.[24,25] Stoichiometry measurements of T252A show a 5% product yield relative to the wild-type, with 51% of the NADH reducing equivalents appearing as hydrogen peroxide and 44% as excess water formation by a four-electron reduction of oxygen (Table I).[26-28] This is the first demonstration of a mutation in the cytochrome P-450s which alters the branching ratio of oxygenase and oxidase activities. In addition, the turnover of camphor by the exogenous oxidant hydrogen peroxide[29-32] is also dramatically reduced. Assuming that the binding and stabilization of hydrogen peroxide is similar to that of dioxygen, the decrease in product yield may be attributed to a disruption in a common mechanism of oxygenase and peroxygenase catalysis. These results demonstrate a crucial role of T252 in P-450 catalysis. X-ray crystallography should provide insight as to whether these effects are due to perturbation of the kinked I-helix, specific chemical alterations of the hydrogen-bonding environment, or availability of protons to aid in dioxygen bond cleavage.

Note Added in Proof. During the proof stage of this manuscript, an independent investigation reporting results with the mutation of threonine 252 was published (Imai, M.; Shimada, M.; Watanabe, Y.; Matsushima-Hibiya, Y.; Makino, R.; Koga, H.; Noriuchi, T.; Ishimura, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 7823-7827). These authors, however, did not consider the possibility of excess water production by the *oxidase* activity of P-450$_{cam}$, and hence did not completely determine the effect of this mutation on the overall stoichiometry.

Acknowledgment. This research was supported by National Institutes of Health Grants GM31756, GM33775, and RRO1811.

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The Dehydrophenyl Anion and the Gas Phase Ion Chemistry of Benzyne

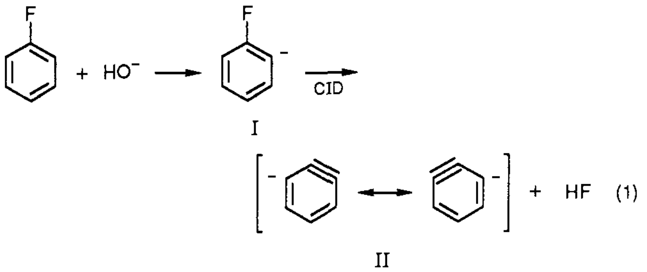
Scott Gronert and Charles H. DePuy*

Department of Chemistry and Biochemistry
University of Colorado, Boulder, Colorado 80309-0215

Received May 8, 1989

We report that in the gas phase the fluorophenyl anion (I) readily undergoes collision-induced dissociation (CID) to form an ion, $C_6H_3^-$, which, on the basis of its chemical reactions, we formulate as the 2,3-dehydrophenyl anion (II). Judging from its relatively weak basicity, II is a highly delocalized species which can be used to investigate the gas phase ion chemistry of its conjugate acid, benzyne, whose reactions closely parallel those in solution.[1]

For the preparation of II we used our tandem flowing afterglow-selected ion flow tube (FA-SIFT).[2] In the source flow tube we generated the fluorophenyl anion (I) by proton abstraction from fluorobenzene. This ion was mass-selected by the SIFT quadrupole and injected into the second flow tube. At low injection potentials, anion I survives intact, but at higher potentials (ca. 50 V),[3] it is completely converted to II by collision-induced dissociation in the helium buffer gas present at 0.5 Torr in the flow tube (eq 1).[4]



An exact measurement of the gas-phase basicity of II is complicated by an apparent kinetic barrier to its protonation as well as by the high reactivity of the benzyne, which is formed once protonation has occurred. For example, when II is allowed to react with CH_3OD, methoxide ion formation is observed in competition with up to three exchanges of hydrogen for deuterium; the overall efficiency of the reaction is 0.3-0.4. II does not abstract a proton from acids weaker than methanol ($\Delta G^{\circ}_{acid} = 374$ kcal mol^{-1}),[5] nor does it exchange with D_2O. We therefore estimate

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(1) For reviews of the solution-phase chemistry of benzyne, see: (a) Grundmann, C. *Houben-Weyl Methoden Der Organishen Chemie*; Grundmann, C., Ed.; Georg Thieme Verlag: Stuttgart, 1981; Band 5,2b, p 613. (b) Hoffman, R. W. *Dehydrobenzene and Cycloalkynes*; Academic Press: New York, 1967. (c) Gilchrist, T. L.; Rees, C. W. *Carbenes, Nitrenes, and Arynes*; Appleton-Century-Crofts: New York, 1969.

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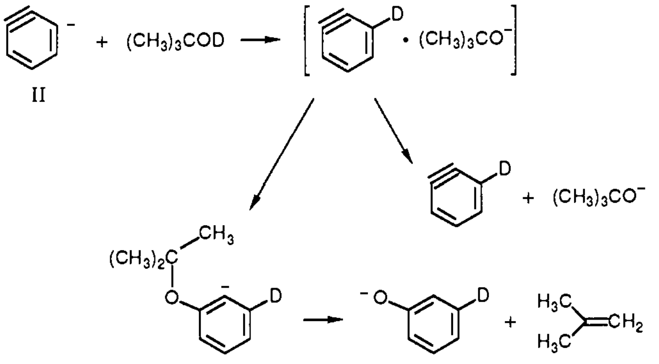
(3) Injection potential refers to the difference in the voltages applied to the source flow tube and the injection plate. The actual kinetic energy of the injected ions is not accurately known.

(4) (a) For a previous example of CID in a SIFT, see: Henchman, M.; Viggiano, A. A.; Paulson, J. F.; Freedman, A.; Wormhoudt, J. *J. Am. Chem. Soc.* **1985**, *107*, 1453. (b) The $C_6H_3^-$ anion has been observed previously in high-energy CID studies of fluoroacetophenone enolates: Harrison, A. G.; Chowdhury, S. K., private communication.

(5) Gas-phase acidities are taken from the following: Lias, S. G.; Bartmess, J. E.; Liebman, J. F.; Holmes, J. L.; Levin, R. D.; Mallard, W. G. *J. Phys. Chem. Ref. Data* **1988**, *17*, Suppl. No. 1.

Scheme I



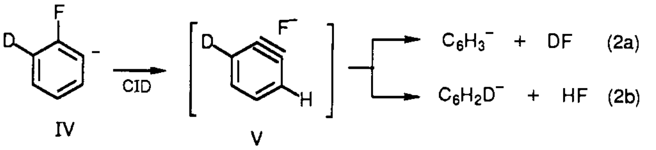
II

III

$\Delta G°_{acid}$(benzyne) = 372 kcal mol^{-1}, 19 kcal mol^{-1} more acidic than benzene and 2 kcal mol^{-1} more acidic than the methyl group in toluene.[6] From this gas-phase acidity and the reported heat of formation of benzyne,[7] we can calculate ΔH_f(II) = 131 kcal mol^{-1}.[8] Since the gas-phase acidities of carbanions correlate well with those in solution,[5] the dehydrophenyl anion could well be a useful synthetic intermediate. In fact, the lithium salt of the dehydrophenyl anion has been postulated as an intermediate in the reaction of 2 equiv of alkyllithiums with aryl sulfones.[9]

With other alcohols, II undergoes exothermic proton transfer with formation of an ion–dipole complex consisting of benzyne and an alkoxide ion, within which further reactions can occur. For example, *tert*-butyl alcohol-*O-d* reacts with II to give $C_6H_4DO^-$, which we formulate as the deuteriophenoxide ion (III), as well as some *tert*-butoxide anion (Scheme I). An analogous process is observed with ethyl and isopropyl alcohol in competition with hydride transfer within the benzyne–alkoxide complex to produce the phenyl anion. Reaction of II with *tert*-butyl mercaptan leads to thiophenoxide ion.

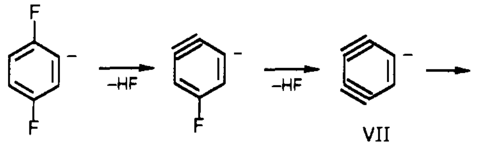
Benzyne is considerably less acidic than HF ($\Delta G°_{acid}$ = 366 kcal mol^{-1}), and it is therefore surprising that II rather than F^- is the nearly exclusive ionic product of the collision-induced dissociation of anion I. Indeed, if HF is added to the flow tube containing II, proton transfer to form F^- and addition to form $C_6H_4F^-$ occur readily. To test for the possible concerted loss of HF, we synthesized *o*-deuteriofluorobenzene, allowed it to react with HO^-, and injected IV (eq 2). When subjected to CID conditions, IV yields nearly equal amounts of deuterated and undeuterated ions, implicating the intermediacy of fluoride-benzyne complex V in the reaction. We do not understand why V does not merely lose F^-. However, simply proposing another structure for II does not eliminate the problem because II, whatever its structure, reacts readily with HF.[10]



IV V

Using ab initio techniques, we have investigated the structure of the 2,3-dehydrophenyl anion calculationally. An optimization at the 6-31+G* level[11] including a frequency analysis indicates that it is a minimum on the potential surface with the ring highly distorted compared to that in benzyne,[12] the angle between the three methynyl carbons being nearly 160°. Such a large geometry change upon protonation could account for its slow proton transfer rate.

Substituted dehydrophenyl anions can also be formed in this way. In particular, the carbanion formed by proton abstraction from *p*-difluorobenzene produces the fluorodehydrophenyl anion (VI). Loss of a second HF molecule occurs readily, to form C_6H^-. Because this ion shows none of the high reactivity of II, we believe an initially formed diynyl anion (VII) undergoes an electrocyclic ring opening to produce a polyacetylide ion, VIII (eq 3). Such a Woodward–Hoffmann allowed transformation to form a stable ion is possible from VII; an analogous reaction of II would produce a vinyl anion. Further experiments designed to shed light on the mechanism of HF loss in these ions are in progress.



VI VII

$$HC\equiv C-C\equiv C-C\equiv C^- \quad (3)$$

VIII

Acknowledgment. We thank Michael Stronach for assistance in some of the experiments and Prof. Alex Harrison for providing unpublished results and participating in helpful discussions. We gratefully acknowledge the support of this work by the National Science Foundation (Grant CHE-8815459).

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(6) The absence of deuterium exchange with D_2O indicates that benzyne is at least 10–15 kcal mol^{-1} more acidic than H_2O ($\Delta G°_{acid}$ = 384 kcal mol^{-1}); see: Grabowski, J. J.; DePuy, C. H.; Van Doren, J. M.; Bierbaum, V. M. *J. Am. Chem. Soc.* **1985**, *107*, 7384.

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(8) Because the proton transfers are slow and it is not practical to carry out bracketing from the reverse direction, our value of $\Delta G°_{acid}$(benzyne) must be termed an estimate and we cannot assign errors. The acidities of the alcohols used in this study have uncertainties of approximately ±2 kcal mol^{-1}.

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(10) Chloride and bromide ions are the exclusive product ions from analogous CID reactions of the chlorophenyl and bromophenyl anions.

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Intramolecular Triplet State Cyclization of But-3-enoxyacetonaphthones

Peter J. Wagner* and Masami Sakamoto

Chemistry Department, Michigan State University
East Lansing, Michigan 48824

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Recently we reported that double bonds attached by a three-atom tether ortho or para on acetophenone quench the ketone triplet[1] and in the process undergo 2 + 2 cycloaddition to the benzene ring,[2] both very efficiently. We now find that 1-butenoxy-2-acetonaphthones and 2-butenoxy-1-acetonaphthones both undergo high-yield internal 2 + 2 cycloadditions from their triplet states, but with much different rate constants. These reactions represent the first examples of 1,2-addition of simple alkenes to triplet naphthalenes. The rate differences suggest significant radical character in the rate-determining step for cyclization.

The isomeric sets of ketones in Scheme I (prepared by standard S_N2 chemistry) produce high chemical yields (70–90% isolated) of the acetylbenzo-2-oxatricyclo[5.4.0.0^{1,5}]undecadienes upon near-UV irradiation in benzene.[3,4] These cyclizations are entirely

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