(2 mM) and phosphate (5 mM). Under these conditions the following internal equilibrium is established at the active site of the enzyme, with 30% of the enzyme-bound material in the form of the intermediate.[4] The intermediate was isolated from the

$$E{\cdot}S3P{\cdot}PEP \rightleftarrows E{\cdot}I \rightleftarrows E{\cdot}EPSP{\cdot}P_i$$

enzyme reaction mixture as described in the legend to Figure 1 and analyzed by NMR.

A proton decoupled ^{31}P NMR spectrum was obtained on the mixture of EPSP (75%) and intermediate (25%). Three major phosphate signals were observed (Figure 1). The two downfield signals correspond to the phosphates in the 3 position of the shikimate ring for both EPSP and the intermediate. A third signal at −4.6 ppm corresponds to the phosphate attached to the tetrahedral carbon of the intermediate. This phosphorus resonance is split into a doublet by coupling to the ^{13}C at the tetrahedral center of the intermediate (see inset), providing definitive assignment.

The proton decoupled ^{13}C NMR spectrum of the purified intermediate (Figure 1) revealed the presence of a single peak having a chemical shift of 101.7 ppm, suggestive of a tetrahedral carbon bearing two oxygens. This resonance was split into a doublet with a coupling constant, $^2J_{CP} = -7$ Hz. This coupling constant was equal, within experimental error (±0.4 Hz), to that observed in the phosphorus spectrum. This carbon–phosphorus coupling demonstrates that the phosphate is attached to the tetrahedral carbon in the intermediate.

A final confirmation of the structure of the intermediate was provided by obtaining a ^1H NMR spectrum (Figure 1). The spectrum was very similar to that previously reported for EPSP,[7] with the addition of a distinctive methyl group of the intermediate at 1.8 ppm that was split into a doublet by coupling to the ^{13}C at the tetrahedral center (see inset).

These observations, coupled to our previous rapid quench kinetics,[4] provide definitive identification of the tetrahedral adduct as a true intermediate in the EPSP synthase reaction pathway. We can now conclude with confidence that the reaction proceeds by an addition–elimination mechanism involving the nucleophilic attack of the 5-OH of S3P on the C-2 of PEP (Scheme I). Although this pathway has been suggested previously,[4,7,8] the current work provides the first unequivocal evidence for an addition–elimination mechanism and lays to rest the controversy surrounding the suggestion of a covalent enolpyruvoyl–enzyme intermediate.[9-11]

Although there are numerous examples of covalent intermediates formed during nucleophilic catalysis, this study provides a unique example of the isolation of a stable, noncovalent enzyme intermediate that is formed and broken down catalytically by the enzyme. This is unusual in that enzymes are most often described by their ability to stabilize an unstable intermediate. Further work is in progress to determine the pH dependence of breakdown of the intermediate in solution and to quantitate fully the mechanistic implications of such an intermediate. Out data suggest that analogues of the tetrahedral intermediate will bind tightly to the enzyme.

Acknowledgment. We thank Professor William P. Jencks for the suggestion that the tetrahedral intermediate should be stable at high pH.

(6) Johnson, K. A. *Meth. Enzymol.* **1986**, *134*, 677–705.
(7) Bondinell, W. E.; Vnek, J.; Knowles, P. F.; Sprecher, M.; Sprinson, D. B. *J. Biol. Chem.* **1971**, *246*, 6191–6196.
(8) Grimshaw, C. E.; Sogo, S. G.; Knowles, J. R. *J. Biol. Chem.* **1982**, *257*, 596–598.
(9) Anton, D. L.; Hedstrom, L.; Fish, S. M.; Abeles, R. H. *Biochemistry* **1983**, *22*, 5903–5908.
(10) Ansano, Y.; Lee, J. J.; Shieh, T. L.; Spreafico, F.; Kowal, C.; Floss, H. G. *J. Am. Chem. Soc.* **1985**, *107*, 4314–4320.
(11) Wibbenmeyer, J.; Brundage, L.; Padgette, S. R.; Likos, J.; Kishore, G. *Biochem. Biophys. Res. Commun.* **1988**, in press.
(12) Abbreviations used are as follows: EPSP, 5-enolpyruvoylshikimate-3-phosphate; glyphosate, *N*-(phosphonomethyl)glycine; HEPES, *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid; S3P, shikimate-3-phosphate; PEP, phosphoenol pyruvate.

Observation of Reactive *o*-Quinodimethanes by Flow NMR

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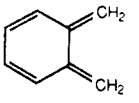
The reactive molecules, *o*-xylylene (**1**),[1] the parent benzenoid *o*-quinodimethane (*o*-QDM), and its derivatives, have been molecules of considerable theoretical[2,3] and experimental[3-7] interest since **1** was first postulated in 1957 by Cava.[8] We have recently



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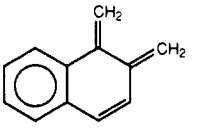
observed several *o*-QDM's by UV–vis spectroscopy and have used the stopped-flow technique to study their rates of dimerization.[9] The first reported ^1H NMR of an *o*-xylylene derivative is that of 2,2-dimethyl-2*H*-indene (2,2-dimethylisoindene) by Dolbier and Michl.[10] In this communication we report the detection of 1,2-dimethylene-1,2-dihydronaphthalene (**2**) and **1** in the presence



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of its stable dimers by the technique of flow ^1H NMR.[11-18] The flow NMR technique allows detection of short-lived species and usefully complements UV–vis techniques because of its diagnostic nature.

Recently, it was reported that *o*-QDM's can be generated by fluoride ion induced 1,4-elimination from [*o*-((trimethylsilyl)-methyl)benzyl]trimethylammonium halides.[7,19] We have found this reaction to be very fast,[9] and thus this method provides an excellent means for the generation of reactive *o*-quinodimethanes under flow NMR conditions because fast, quantitative formation of the transient species is desired.

Our flow NMR apparatus was patterned after that of Fyfe[11,13-18,20] who has pioneered the use of flow NMR in the detection and characterization of reactive intermediates. Because successful detection of a reactive intermediate requires the proper

(1) McCullough, J. J. *Acc. Chem. Res.* **1980**, *13*, 270.
(2) (a) Baudet, J. *J. Chim. Phys. Physichochim. Biol.* **1971**, *68*, 191. (b) Baird, N. C. *J. Am. Chem. Soc.* **1972**, *94*, 4941.
(3) (a) Michl, J.; Flynn, C. R. *J. Am. Chem. Soc.* **1973**, *95*, 5802. (b) Michl, J.; Flynn, C. R. *J. Am. Chem. Soc.* **1977**, *96*, 3280.
(4) Roth, W. R.; Scholz, B. P. *Chem. Ber.* **1981**, *114*, 374.
(5) Oppolzer, W. *Synthesis* **1978**, 793.
(6) Nicolau, K. C.; Barnette, W. E.; Ma, P. *J. Org. Chem.* **1980**, *45*, 1463.
(7) Ito, Y.; Nakatsuka, M.; Saegusa, T. *J. Am. Chem. Soc.* **1982**, *104*, 7609.
(8) Cava, M.; Napier, D. R. *J. Am. Chem. Soc.* **1957**, *79*, 1701.
(9) Trahanovsky, W. S.; Macias, J. R. *J. Am. Chem. Soc.* **1986**, *108*, 6820.
(10) Dolbier, W. R., Jr.; Kazumasa, M.; Michl, J.; Horák, D. V. *J. Am. Chem. Soc.* **1977**, *99*, 3876.
(11) (a) Fyfe, C. A.; Cocivera, M.; Damji, S. W. H. *Acc. Chem. Res.* **1978**, *11*, 277 and references cited therein. (b) Selivanov, S. I.; Ershov, B. A. *Russian Chem. Rev.* **1986**, *55*, 395 and references cited therein.
(12) Malatesta, V.; Cocivera, M. *J. Org. Chem.* **1978**, *43*, 1737.
(13) Fyfe, C. A.; Damji, S. W. H.; Koll, A. *J. Am. Chem. Soc.* **1979**, *101*, 951.
(14) Fyfe, C. A.; Damji, S. W. H.; Koll, A. *J. Am. Chem. Soc.* **1979**, *101*, 956.
(15) Damji, S. W. H.; Fyfe, C. A. *J. Org. Chem.* **1979**, *44*, 1757.
(16) Cocivera, M.; Effio, A. *J. Org. Chem.* **1980**, *45*, 415.
(17) Tan, L. K.; Cocivera, M. *Can. J. Chem.* **1982**, *60*, 772.
(18) Tan, L. K.; Cocivera, M. *Can. J. Chem.* **1982**, *60*, 778.
(19) Ito, Y.; Nakatsuka, M.; Saegusa, T. *J. Am. Chem. Soc.* **1980**, *102*, 863.
(20) Bruck, D.; Dudley, R.; Fyfe, C. A.; Van Delden, J. *J. Magn. Reson.* **1981**, *42*, 51.

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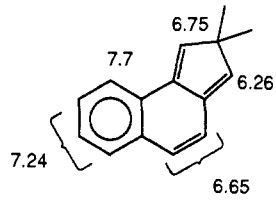
balance of the rates of a number of processes, we designed the apparatus to allow us to vary flow rates by changing the transfer and detector volumes and to be mechanically reliable so that we could carry out many runs varying conditions to achieve success. The apparatus was designed to be easily inserted and removed from the probe of a Bruker WM-300 pulse FT NMR spectrometer.[21,22]

We have determined by UV–vis spectroscopy that **2** is ca. 100 times less reactive than **1**[24] and indeed we can obtain the ^{1}H NMR of **2** before it dimerizes. The NMR signals of solutions formed by mixing an acetonitrile-d_3 (CD_3CN) solution of **3**[25] (0.01 M) and a CD_3CN solution of tetrabutylammonium fluoride (TBAF)[9]

3 **2**

(0.166 M) at flow rates of 1–24 mL/min were detected. The spectrum obtained at a flow rate of 12 mL/min, Figure 1a, is consistent with that expected for **2**. The low field portion of the spectrum is similar to that of **4**,[27] and the δ 6 to 5 region shows



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four exocyclic methylene proton signals, one of which is higher than the others because the corresponding proton is not deshielded by an adjacent π bond. At faster flow rates, signals for **3** were observed, and at slower flow rates, signals for the dimers[24] of **2** were observed.

The high reactivity of **1** prevented us from obtaining its spectrum in the absence of its dimers. The NMR spectra of solutions formed by mixing CD_3CN solutions of [*o*-((trimethylsilyl)-methyl)benzyl]trimethylammonium iodide (**5**)[9] (10^{-3} M) and TBAF[9] (6×10^{-3} M) at flow rates of 3–45 mL/min were obtained. At slow flow rates, the low field part of the spectrum showed only the [4 + 2] and [4 + 4] dimers of **1** in a ratio of 9:1, the expected products and ratio.[9] These spectra looked very similar to those of the recycled product mixture (see Figure 1b). At higher flow rates, this part of the spectrum showed four additional signals (see Figure 1 (parts c and d)) which we have assigned to the protons of **1**: δ 6.32 (α), 6.00 (β), 5.66 ((*Z*)-methylene), and 5.00

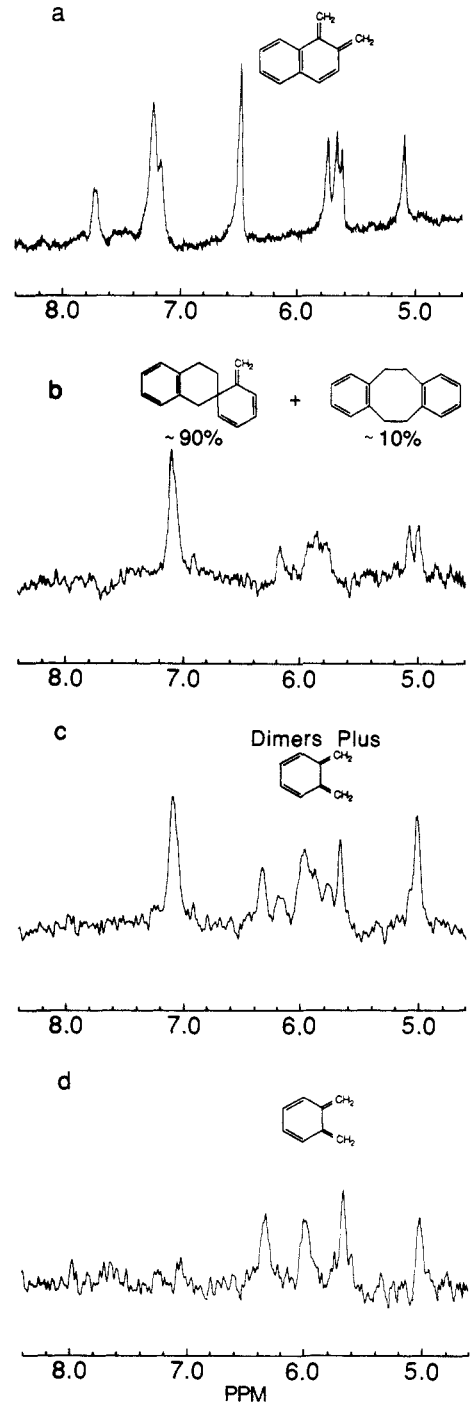


Figure 1. 300 MHz ^{1}H NMR spectra: (a) formed by mixing 0.01 M **3** in CD_3CN with 0.166 M TBAF in CD_3CN; flow rate, 12 mL/min; number of scans, 500; pulse interval, 1 s; (b) of the recycled product mixture resulting from mixing 10^{-3} M **5** in CD_3CN with 6×10^{-3} M TBAF in CD_3CN; flow rate, 45 mL/min, number of scans, 1293; pulse interval, 0.127 s; (c) formed by mixing 10^{-3} **5** in CD_3CN with 6×10^{-3} M TBAF in CD_3CN; flow rate, 45 mL/min; number of scans, 831; pulse interval, 0.127 s; (d) computer subtraction of spectrum b from spectrum c.

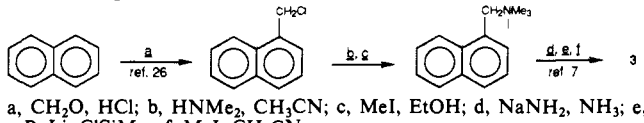
(21) A detailed description of the apparatus will be presented in a full paper. The two reactant solutions are flowed into a Teflon mixing chamber constructed as previously described.[23] The flow tube was constructed from a 5-mm NMR tube and is connected to the mixing chamber by a glass-to-Teflon union. A capillary tube fused inside the NMR tube delivers the mixed solution to the bottom of the NMR tube which is in the detector coil. The mixed solution flows upward and out of the tube through a side arm. The mixing chamber is attached to a Teflon base. The precursor of the reactive intermediate is magnetized before mixing by passing the solution of the precursor through a coil of polyethylene tubing contained in a cavity inside the base before it enters the mixing chamber. The flow of the solutions is controlled by a dual syringe pump. Shortly before obtaining spectra, the mixing chamber-flow tube-base assembly is lowered into the probe of the spectrometer. The two syringes are connected to the assembly by two 6 foot pieces of polyethylene tubing. A third 6 foot piece of tubing carries the mixed solution from the flow tube to a collection flask.

(22) Special problems associated with the use of pulsed FT-NMR for the observation of reactive species are discussed in the following: Kühne, R. O.; Schaffhauser, T.; Wokaum, A.; Ernst, R. R. *J. Magn. Reson.* **1979**, *35*, 39.

(23) Fyfe, C. A.; Cocivera, M.; Damji, S. W. H.; Hostetter, T. A.; Sprout, D.; O'Brien, J. *J. Magn. Reson.* **1976**, *23*, 377.

(24) Macias, J. R. Ph.D. Dissertation, Iowa State University, Ames, IA, 1987.

(25) Compound **3** was prepared by the following sequence and ^{1}H NMR, and the exact mass (obtained by the Fast Atom Bombardment technique) of the cation ($RNMe_3^+$) and the salt and iodide ion ($RNMe_3I_2^-$) are consistent with the assigned structure:



a, CH_2O, HCl; b, $HNMe_2$, CH_3CN; c, MeI, EtOH; d, $NaNH_2$, NH_3; e, *n*-BuLi, $ClSiMe_3$; f, MeI, CH_3CN.

(26) Grummitt, O.; Burle, A. *Organic Syntheses*, Wiley: New York, Collect. Vol. III, p 195.

(27) Dolbier, W. R., Jr.; Dulcere, J.-P.; Sellers, S. F.; Koroniak, H.; Shatkin, B. T.; Clark, T. L. *J. Org. Chem.* **1982**, *47*, 2298.

((*E*)-methylene). These assignments are in agreement with the signals expected for **1** on the basis of the spectrum of **2**. These signals clearly result from a reactive intermediate because they decrease as the flow rate is reduced.

Theoretical[2] and experimental[3] work has postulated that **1** exists as a singlet ground state. Our observation of **1** by flow ^{1}H NMR confirms this.

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Oxygen Isotope Exchange between Water and Semiquinones

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The no-spin oxygen atoms in the anion radicals of carbonyl systems are readily replaced by ^{17}O with its 5/2 nuclear spin by the simple addition of ^{17}O labeled water to the anion radical solutions in hexamethylphosphoramide (HMPA) or liquid ammonia. This reaction can be utilized to readily produce ^{17}O labeled anion radicals that yield strong well resolved ESR signals exhibiting splitting from the ^{17}O nucleus. This very simple procedure makes ^{17}O substituted carbonyl anion radicals for spin density,[1] ion association,[2] hydrogen bonding,[3] etc. studies readily available without necessitating the synthesis of the isotopically substituted precursors.

When water is added to a solution of the indane-1,2,3-trione (ninhydrin) anion radical in HMPA the formation of the hydrogen bonding between the water and the anion radical results in a decrease in the proton coupling constants (A_H = 0.93 G, 2 H's and A_H = 1.18 G, 2 H's), which is well documented.[4] However, when 5 μL (0.30 mmol) of 20% $H_2{}^{17}O$ is added to 1.0 mL of a ca. 10^{-3} M solution of the ninhydrin anion radical in HMPA,[4] the expected changes in the coupling constants are accompanied by the nine line spectrum being slowly replaced with that of the ^{17}O substituted (A_O = 3.99 G) anion radical. The intensity of the ^{17}O substituted anion radical continues to grow for a period of about 30 h, and the two spectra can be observed simultaneously without apparent loss of total anion radical concentration, Figure 1. When the same reaction is carried out with a solution containing 0.1 M neutral triketone, the reaction is complete within 10 min. The anion radical solutions generated in the presence of excess neutral ninhydrin exhibit large line widths due to rapid electron exchange. This together with the coupling constant changes due to hydrogen bonding reduces the nine line pattern to five resolvable ESR lines. After several hours, ESR analyses of these solutions clearly show the presence of the anion radical containing two ^{17}O atoms.

Scheme I

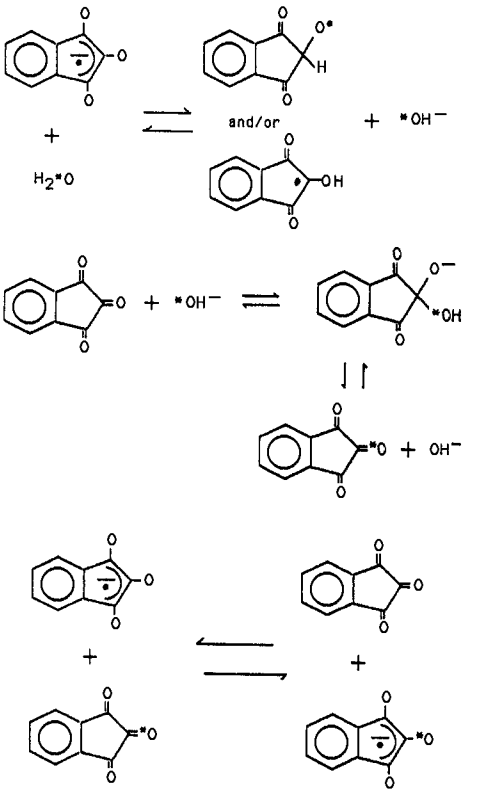

Table I. Oxygen-17 Hyperfine Splitting Constants in Gauss in DMF at 294 K[a]

anion radical	A_0 (DMF)	A_0 (solv, temp (K))
ninhydrin	unknown	3.99 (HMPA, 298)
benzoquinone	9.53	9.42 (NH_3, 205)
anthraquinone	7.53	7.32 (NH_3, 205)
		7.47 (HMPA, 298)
fluorenone	9.21	9.54 (HMPA, 298)

[a] Taken from ref 1 and those measured in this work.

It is interesting that only one ^{17}O coupling constant for $C_9H_4O_2{}^{17}O^{\bullet-}$ is observed despite the fact that there are two possible sites of substitution. This is, however, the expected result since the rates of isotopic substitution are probably not the same at the two different sites. Even if they were, LCAO calculations predict nearly the same spin and charge densities on all three oxygen atoms.[5]

The fact that the hydroxide ion will react with anthraquinone ($C_{14}H_8O_2$) to form the hydroxide addition complex[6] ($C_{14}H_8O_2$-OH^-) coupled with the strong dependence of the rate of ^{17}O substitution into the ninhydrin anion radical upon the neutral molecule concentration provides strong evidence that the reaction proceeds via the initial formation of the hydroxide ion followed by the formation of the hydroxide addition complex, Scheme I. That is, the anion radical acts as a base strong enough to deprotonate water, and the hydroxide addition must be followed by electron exchange.

The reaction appears to be quite general in nature; a strong well resolved ESR spectrum of benzoquinone-$^{17}O^{\bullet-}$ and anthraquinone-$^{17}O^{\bullet-}$ can be obtained in the absence of excess neutral molecule several hours after the addition of 5 μL of $H_2{}^{17}O$ to 1 mL of the anion radical solutions in HMPA or liquid ammonia. In HMPA, these anion radicals are free of ion association.[7] Thus, the ^{17}O coupling constants are unperturbed by interaction of the

(1) Oxygen-17 hyperfine coupling constants are a sensitive function of the σ–π interaction, see: Broz, M.; Luz, Z. *J. Chem. Phys.* **1969**, *51*, 738.

(2) The carbonyl oxygen atoms represent the site of anion radical–gegenion interaction in ion pairs involving ketyls, semidiones, and semiquinones, see for example: (a) Nakamura, K. *J. Am. Chem. Soc.* **1080**, *102*, 7847. (b) Felix, C. C.; Sealy, R. C. *J. Am. Chem.* Soc. **1982**, *104*, 1555. (c) Stevenson, G. R.; Alegria, A. E.; McB. Block, A. *J. Am. Chem. Soc.* **1975**, *97*, 4859.

(3) Hydrogen bonds with ketyls involve the carbonyl oxygen, see: Ichikawa, T.; Ichikawa, Y.; Yoshida, H. *J. Phys. Chem.* **1988**, *92*, 508.

(4) Alegria, A. E.; Fontanez, F.; Stevenson, G. R. *J. Phys. Chem.* **1976**, *80*, 1113.

(5) Lasia, A.; Kalinowski, M. K. *J. Electroanal. Chem.* **1972**, *36*, 511.

(6) Roberts, J. L.; Sugimoto, H.; Barrette, W. C.; Sawyer, D. T. *J. Am. Chem. Soc.* **1985**, *107*, 4556.

(7) (a) Levin, G.; Jaugar-Grodzinski, J.; Szwarc, M. *J. Am. Chem. Soc.* **1970**, *92*, 2268. (b) Stevenson, G. R.; Alegria, A. E. *J. Phys. Chem.* **1974**, *78*, 1771.

0002-7863/88/1510-6581$01.50/0