limited while its decay lifetime is ~230 ns, in good agreement with the value reported by Scaiano and Abuin.[3]

Detection along Y should give initially a signal due to the interacting pair, which decays via reactions 3 and 4 and subsequently the "growing in" of the free radicals formed via 4. Figure 3a shows the Y kinetic trace. Within experimental error, the Y rise time matches the decay of the X signal. This is expected since $k_{ISC}$ and $k_{ESC}$ are of similar magnitude, while reaction 4 is the main radical-pair decay route in our field of ~3500 G.

According to the above interpretation, the decay rate of the X signal is completely uncoupled from the intersystem crossing rate since the only radical pairs that contribute to the X signal are those that exist as pairs when the first $\pi/2$ microwave signal is applied but have produced a free radical at the time that the echo is observed. In the framework of this model, therefore, $k^X_{obsd} = k_{ESC}$ while $k^Y_{obsd} = k_{ESC} + k_{ISC}$.

Although the signal to noise ratio for the Y signal shown in Figure 1b is rather poor, it is reproducible and can be interpreted as being due to a spin-polarized triplet radical pair with zero field splitting parameters $D \simeq 0.001$ cm$^{-1}$, $E = 0$ cm$^{-1}$, giving by the point dipole approximation an average distance between the radicals of ~14 Å.[10] The polarization pattern (emission, enhanced absorption) is that expected for population of the pair from the spin-polarized $^3$BP[11] and decay from the $T_0$ level of the radical pair. EPR spectra (Y signal) taken at longer delay times with respect to the laser (not shown) show the BP ketyl radical completely in emission.

In conclusion, this application of time-resolved ESE to photoreactions in micelles supports the mechanism proposed to explain a time-dependent echo phase shift. It also demonstrates that these methods can be used to obtain directly the rate of radical escape from micelles and observe directly radical-pair interactions within the micelle.

**Acknowledgment.** Fruitful discussion with Drs. A. Trifunac, J. Norris, and R. Lawler are gratefully acknowledged.

**Registry No.** Benzophenone, 119-61-9; 1,4-cyclohexadiene, 628-41-1; sodium dodecylsulfate, 151-21-3.

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## $^{13}$C NMR Evidence of Carbinolamine Formation at the Active Site of an Imine-Forming Aldolase[1]

Bruce David Ray[2] and Edwin T. Harper*

*Department of Biochemistry*
*Indiana University School of Medicine*
*Indianapolis, Indiana 46223*

Wilmer K. Fife

*Department of Chemistry*
*Indiana University—Purdue University at Indianapolis*
*Indianapolis, Indiana 46223*

*Received January 20, 1983*

In a number of biochemical reactions, carbon–hydrogen and carbon–carbon bonds are activated toward cleavage by conversion of adjacent carbonyl groups to protonated imines, which often can be detected by reductive trapping in the presence of sodium borohydride. The aldol cleavage of ketose phosphates by mammalian fructose 1,6-bisphosphate aldolase (EC 4.2.1.13) is a case in point.[3] Since carbinolamines invariably occur as intermediates in nonenzymic interconversions between carbonyl compounds and imines,[4] it may be reasonable to expect that imine-forming enzymes will also form hitherto undocumented enzyme–substrate carbinolamine intermediates. We now report that the predominant form of the aldolase–glycolaldehyde phosphate complex is an enzyme-stabilized carbinolamine.

Isotopically enriched (90% $^{13}$C) D,L[2-$^{13}$C]glyceraldehyde was synthesized by adaptations of the methods of Serianni et al.,[5] and was converted to [1-$^{13}$C]glycolaldehyde phosphate (**1**) and [1-$^2$H,1-$^{13}$C]glycolaldehyde phosphate (**2**) by methods that will be described elsewhere.[6,7] Aldolase was purified from rabbit muscle by the method of Penhoet et al.[8] to a specific activity of 15 units/mg. For spectroscopic studies, aldolase preparations were freed of triose phosphate isomerase activity by treatment with glycidol phosphate[9] and concentrated to 120 mg/mL by precipitation with ammonium sulfate, dialysis, and ultrafiltration. Heavy-metal ions were removed on Chelex-100 (Bio-Rad Laboratories). Spectra were obtained at 8 °C on Nicolet NT-150 or NT-200 NMR (nuclear magnetic resonance) spectrometers operating in the FT mode with quadrature detection over spectral widths of 4500 or 5500 Hz, respectively. Proton decoupling was achieved by 10-W irradiation, during data acquisition only (370 ms/pulse), at 150.0577 or 200.067 15 MHz, respectively. A standard single-pulse sequence was used, with a 1-s delay between pulses and a flip angle of 37°. Chemical shifts were calculated relative to that of an internal standard (dioxane, 67.4 ppm).

The $^1$H-decoupled $^{13}$C NMR spectrum of **1** showed a doublet at 89.9 ppm ($^3J_{CP}$ = 7.9 Hz), which was assigned to the $CH(OH)_2$ group of the aldehyde hydrate. No signals were detected in the region near 200–210 ppm, indicating that the aldehyde was at least 97% hydrated. The $^{13}$C NMR spectrum of **2**, measured without proton decoupling, consisted of a triplet (89.5 ppm, $^1J_{CD}$ = 21.8 Hz) as well as a doublet (90.2 ppm, $^1J_{CH}$ = 153.8 Hz), each peak of which represented a poorly resolved doublet arising from $^{13}$C–$^{31}$P coupling. The relative areas of the triplet and the doublet indicated that **2** was 75% deuterated at the aldehyde carbon.

The structure of the aldolase–**2** complex was indicated by the spectrum shown in Figure 1A, compared to that of the enzyme alone (Figure 1B). The striking observation was that the single resonance unique to the complex appeared at 79.7 ppm (shaded peak in Figure 1A), in a region characteristic of sp$^3$- rather than sp$^2$-hybridized carbon. The resonance was shown to arise from bound **2** by reappearance[10] of the doublet resonance of free **2** upon displacement by excess (26 mM) hexitol 1,6-bisphosphate.[11] The relative areas (0.06) of the peaks at 79.5 (residual bound **2**) and 87.1 ppm (free **2**)[10] permitted calculation of a dissociation constant (1.3 $\mu$M) of the aldolase–**2** complex that is consistent with that (2.5 $\mu$M) of the unlabeled complex.[12]

The chemical shift of the new resonance showed that **2** was predominantly bound either as a noncovalent complex of the aldehyde hydrate or as a covalent carbinolamine intermediate, but not as an imine.[13] The complex was identified as a carbi-

(1) Reported in part at the 66th Annual Meeting of the Federation of American Societies for Experimental Biology, New Orleans, LA, Apr 1982.
(2) Present Address: Department of Physics, Indiana University—Purdue University at Indianapolis, IN 46223.
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(10) The resonance reappeared at 87.1 ppm, perhaps due to fast exchange between free and nonspecifically bound forms.
(11) Prepared by the following procedure: Ginsburg, A.; Mehler, A. H. *Biochemistry* **1966**, *5*, 2623–2634.
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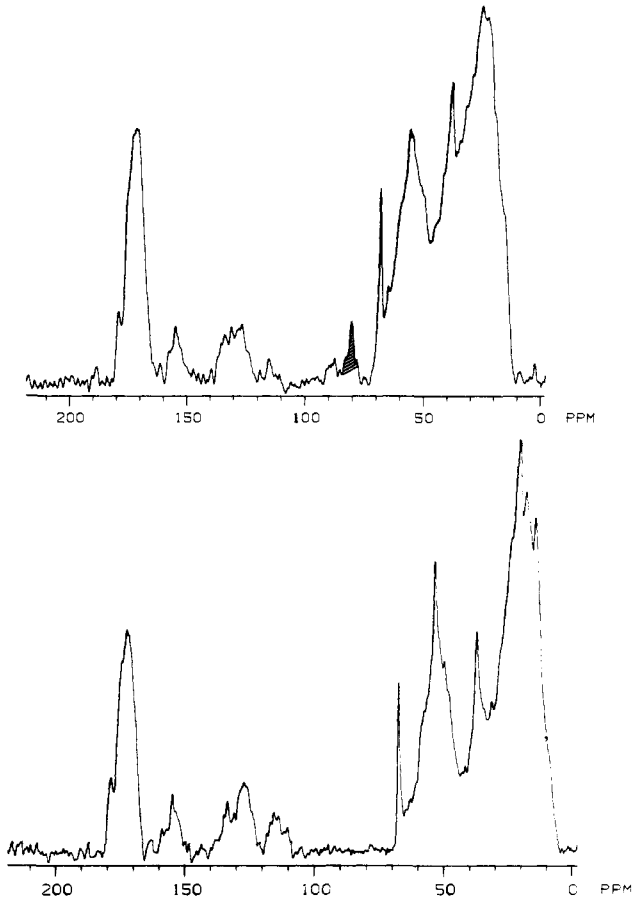


**Figure 1.** (A, top) Proton-decoupled 50-MHz $^{13}C$ NMR spectrum (4000 scans) of 110–112 mg/mL aldolase (2.8 mM subunits) in the presence of 3.6 mM [1-$^{2}H$,1-$^{13}C$]glycolaldehyde phosphate (**2**) in 5 mM triethanolamine·HCl buffer, pH 7.4–7.7, at 8 °C. Sharp peaks at 67.4 and 53.3 ppm represent dioxane and buffer, respectively. (B, bottom) Spectrum of the enzyme alone (12 000 scans).

nolamine rather than a hydrate by the following considerations. First, the 9.8-ppm change in chemical shift upon binding of **2** appears to be too large for a simple change in the environment of the hydrate due to noncovalent binding. In the interaction of hydroxyacetone phosphate with aldolase, which does appear to be predominantly noncovalent,[14] chemical shifts of carbonyl and hydrate resonances were altered by no more than 0.1 ppm.[6] Second, the dissociation constant of glycolaldehyde phosphate is comparable to that of dihydroxyacetone phosphate, which is known to form predominantly covalent complexes, whereas noncovalent binding of monophosphate esters is typically associated with dissociation constants that are 2–3 orders of magnitude larger.[14,15] Third, the chemical shift of the resonance of bound **2** changes by 0.5 ppm between pH 7.1 and 9.4,[6] which is within the observed range of pH effects on $\alpha$-carbon chemical shifts of protonated and unprotonated alkylamines.[16]

The enzyme-bound carbinolamine is presumably stabilized by interactions with amino acid residues at the active site, because carbinolamines do not predominate in the nonenzymic reaction of glycolaldehyde phosphate with amines.[17] Since similar interactions may stabilize carbinolamine-like transition states at the active site, the residues may also play a catalytic role in the interconversion between free substrate and enzyme-bound imine. It is interesting that formation and breakdown of an aldolase–dihydroxyacetone phosphate imine appears to be at least partially rate limiting in the overall reaction of fructose 1,6-bisphosphate.[15a,c] This suggests that the aldolase–glycolaldehyde phosphate carbinolamine may represent a transition-state analogue of the overall reaction, provided that glycolaldehyde phosphate and dihydroxyacetone phosphate interact with the enzyme by the same catalytic mechanism, and may account for the tight binding of the partial substrate, glycolaldehyde phosphate.

**Acknowledgment.** This research was supported in part by the Grace M. Showalter Foundation. The NMR experiments were carried out at the Purdue University Biochemical Magnetic Resonance Laboratory, which is supported by NIH Grant RR-01077. We thank the laboratory staff for patient instruction and assistance. We thank Prof. B. D. Nageswara Rao and Dr. E. L. Ferroni for helpful discussions.

**Registry No. 1**, 85710-91-4; **2**, 85710-92-5; fructose 1,6-bisphosphate aldolase, 9024-52-6.

(13) Inactivation of the complex by borohydride ion implies that some imine is in relatively rapid equilibrium with the carbinolamine. The noise level of the spectrum in Figure 1A (carbinolamine S/N = 8.1) could mask as much as 34% of imine, if it were represented by an undetectable resonance (S/N <2) with a line width equal to that of the carbinolamine resonance. The possibility that a larger quantity of imine might have been concealed by a broader resonance was considered unlikely because (a) the ratio of peak areas of $^{13}C$-enriched and protein carbonyl resonances in Figure 1A (0.039) was comparable to that (0.042) in the spectrum of a borodeuteride-reduced aldolase–[2-$^{13}C$]dihydroxyacetone phosphate complex;[6] (b) no hidden broad bands were revealed by apodization of the free-induction decay signal of Figure 1A with progressively larger line widths (≤150 Hz).

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(17) Aqueous solutions containing 3.3 mM **1** and excess butylamine or (2,2-dimethoxyethyl)amine (pH > p$K_a$), exhibited resonances due to enriched carbon at 165–170 ppm ($^{3}J_{CP}$ = 8.4 Hz; imine) and 90.3 ppm ($^{3}J_{CP}$ = 7.3 Hz; hydrate). Resonances attributable to carbinolamine (70–90 ppm) were not seen at any pH value.

## Synthetic Studies on the Taxane Diterpenes. Utility of the Intramolecular Diels–Alder Reaction for a Single-Step Stereocontrolled Synthesis of a Taxane Model System

Kunio Sakan*

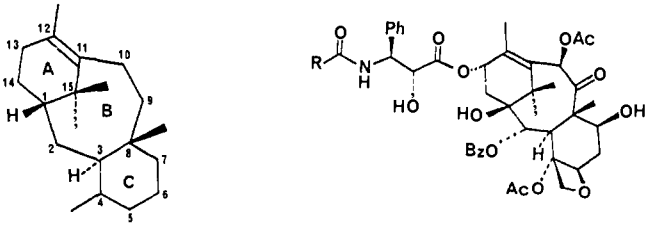
*Department of Chemistry, Carnegie-Mellon University Pittsburgh, Pennsylvania 15213*

Bryan M. Craven*

*Department of Crystallography, University of Pittsburgh Pittsburgh, Pennsylvania 15260*

*Received February 7, 1983*

The taxane diterpenes,[1] isolated from various species of *Taxus*, possess the unusual tricyclic carbon framework **1**[2] containing a




**1**

**1a** $\Delta^{4,5}$-9-oxo-derivative of **1**

**2** R = Ph

**3** R = CMeCHMe

sterically congested eight-membered B ring. Some of these such as taxol[3] (**2**) and cephalomannine[4] (**3**) exhibit highly promising

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