Stereospecific Claisen Rearrangement Catalyzed by an Antibody†

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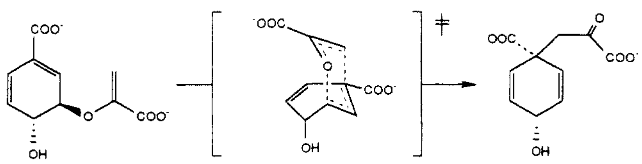
Rate accelerations, regiospecificity, and stereoselectivity are the hallmarks of enzyme-catalyzed reactions. In the construction of artificial enzymes, the exacting specificity of biological catalysts is perhaps the most important and difficult property to mimic. It is therefore significant that an antibody-catalyzed reaction, the lactonization of a δ-hydroxy ester, is enantioselective.[1] We recently reported[2] catalysis of another class of reaction by an immunoglobulin: a monoclonal antibody, elicited against the transition-state analogue inhibitor **3** for chorismate mutase, significantly accelerates the Claisen rearrangement of chorismate (**1**) to prephenate (**2**). We now wish to report that this regioselective catalysis by an antibody is also highly stereospecific.



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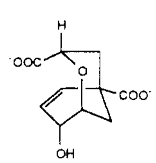

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Hybridoma 1F7 was propagated in ascites as previously described.[2] Monoclonal antibody was purified to apparent homogeneity, as judged by SDS-PAGE with Coomassie staining,[3] by a two-step procedure involving affinity chromatography on a column of immobilized protein A[4] followed by FPLC ion-exchange chromatography on a Mono Q HR 10/10 column (Pharmacia).[5] Protein concentration was estimated by the method of Smith et al.[6] An enzyme-linked immunosorbent assay (ELISA)[7] verified the high affinity of the monoclonal for the oxabicyclic transition state analogue **3**.

The purified immunoglobulin was assayed with (−)- and (±)-chorismate.[8] Under conditions in which all of the (−)-isomer rearranges to product, the racemate shows biphasic kinetics (Figure 1). Extrapolation of the slow phase back to zero time shows that approximately half of the (±)-isomeric mixture is consumed in the fast reaction. The Michaelis parameters for the two materials were determined by the method of initial rates in phosphate-buffered saline (pH 7.5) at 13 °C. The apparent values of k_{cat} and K_m for (−)-chorismate are 0.025 ± 0.001 min^{-1} and 22 ± 2 μM, in reasonable agreement with the previously determined values.[2] Although the value of k_{cat} for the racemate is the same (0.023 ± 0.003 min^{-1}), its apparent K_m value is two times larger (44 ± 7 μM). Together, these results suggest that the (+)-isomer of chorismate is not a substrate for the catalytic antibody.

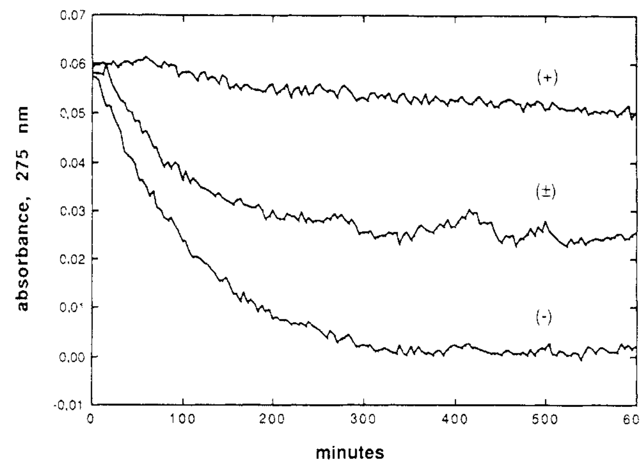

Figure 1. Rearrangement of (−)-chorismate, (±)-chorismate, and (+)-chorismate with monoclonal antibody 1F7. Antibody (18 μM) was incubated with substrate (ca. 20 μM) in phosphate-buffered saline (pH 7.5) at 14 °C. Reaction was monitored spectroscopically at 275 nm.

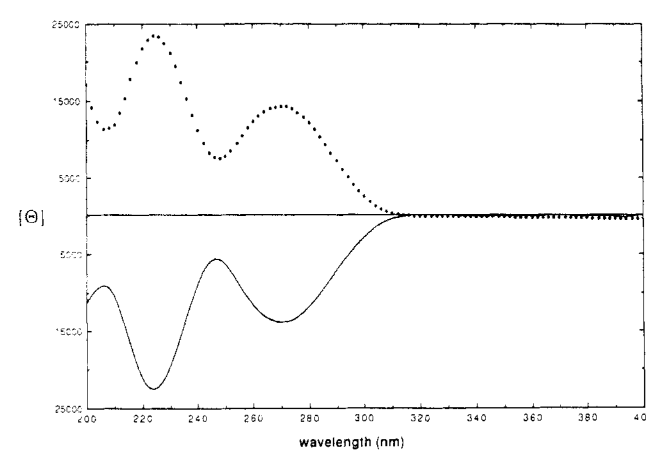

Figure 2. Circular dichroism spectra of authentic (−)-chorismate (—) and kinetically resolved (+)-chorismate (···). The spectra were measured in deionized water (5 scans, 14 °C) on an Aviv 60DS CD spectrophotometer.

An authentic sample of (+)-chorismate was obtained by kinetic resolution of the racemate. (±)-Chorismate (75 μM) was reacted with the catalytic antibody (18 μM) at 24 °C for 6.5 h. The reaction mixture was ultrafiltered at 4 °C (Centricon 30, Amicon) to remove protein and concentrated by lyophilization. Unreacted chorismate was isolated by preparative HPLC and shown to coelute with an authentic sample of (−)-chorismate by analytical HPLC. Comparison of the circular dichroism spectrum (Figure

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(8) (±)-Chorismate was synthesized from racemic methyl (1β,6β)-5β-[[1-(methoxycarbonyl)ethenyl]oxy]-7-oxabicyclo[4.1.0]hept-3-ene-3-carboxylate, generously provided by Drs. Glenn Berchtold and Robert Padykula, in three steps according to a literature procedure: Hoare, J. H.; Policastro, P. P.; Berchtold, G. A. *J. Am. Chem. Soc.* **1983**, *105*, 6264-6267. Racemic chorismate and the natural (−)-isomer (Sigma) were purified by preparative HPLC on a Vydac C-18 218-TP-510 reverse phase column (10 mm × 25 cm, 6 mL/min, isocratic elution with 93% aqueous TFA (0.05%)/7% CH_3CN).

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2) of the isolated material with that of the natural isomer verified that the former was optically pure (+)-chorismate.

As shown in Figure 1, (+)-chorismate is a poor substrate for the catalytic antibody. The ratio of the initial rates of rearrangement obtained for the (−)- and (+)-isomers, corrected for the spontaneous background reaction, is 38 under the depicted conditions. Extrapolation to lower substrate concentrations (well below K_m for (−)-chorismate) provides an estimate of the enantioselectivity of the catalyst closer to 90:1.

In summary, we have shown that an induced antibody with chorismate mutase activity possesses exquisite enzyme-like specificity. Our findings are significant as they further demonstrate the potential of these tailored catalysts for chiral discrimination on a practical level. Since strain and proximity are the principal catalytic effects antibodies are likely to impart, we are currently targeting other shape-selective reactions, especially sigmatropic rearrangements and Diels–Alder cyclizations, with the expectation that any catalytic antibodies generated will exert precise regio- and stereochemical control over the promoted transformations.

Acknowledgment. We are grateful to Drs. Glenn Berchtold and Robert Padykula for the generous gift of racemic methyl (1β,6β)-5β-[[1-(methoxycarbonyl)ethenyl]oxy]-7-oxabicyclo-[4.1.0]hept-3-ene-3-carboxylate. We also thank Stephen Carpenter and Maria-Theresa Auditor for performing some preliminary experiments. This work was supported in part by a Junior Faculty Research Award to D.H. from the American Cancer Society and Grant GM38273 from the National Institutes of Health (D.H.).

Local Structure Evaluation in Solid Organophosphorus Compounds by Double Cross Polarization Carbon-13 Nuclear Magnetic Resonance Spectroscopy[†,‡]

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Atom connectivity information that yields molecular structure descriptions is inaccessible in conventional solid-state ^{13}C NMR spectroscopy. The ^{1}H–^{13}C–^{31}P double cross polarization (DCP)/MAS ^{13}C NMR experiment reported here uses the direct dipolar interaction between isolated ^{13}C–^{31}P spin pairs in an organic solid to identify the subset of carbons within a spherical volume element of 0.4 nm radius centered on the ^{31}P atom. These chemical shift-labeled carbons are further delineated by their ^{31}P–^{13}C cross polarization rates which encode ^{31}P–^{13}C internuclear distances. Hence, the experiment reveals the carbon types in the first, second, and third bonding spheres with respect to the phosphorus atom, furnishing a statistical description of the carbon bonding network at this site.

The essence of this experiment is contained in the ^{13}C–^{31}P cross polarization step. The ^{13}C–^{31}P dipolar interaction acts as a selective filter within the molecular framework that restricts the ^{13}C spectrum to the resonances of those carbons that acquire magnetization by transfer from ^{31}P. The experiment is performed in such a way that the ^{13}C signal intensity accrues by the ^{31}P–^{13}C cross polarization rate, $(T_{CP})^{-1}$, and decays by $(^{13}CT_{1\rho})^{-1}$.[1] The relative magnitude of these rates determines the effective radius of the volume element in which DCP signals can be observed.

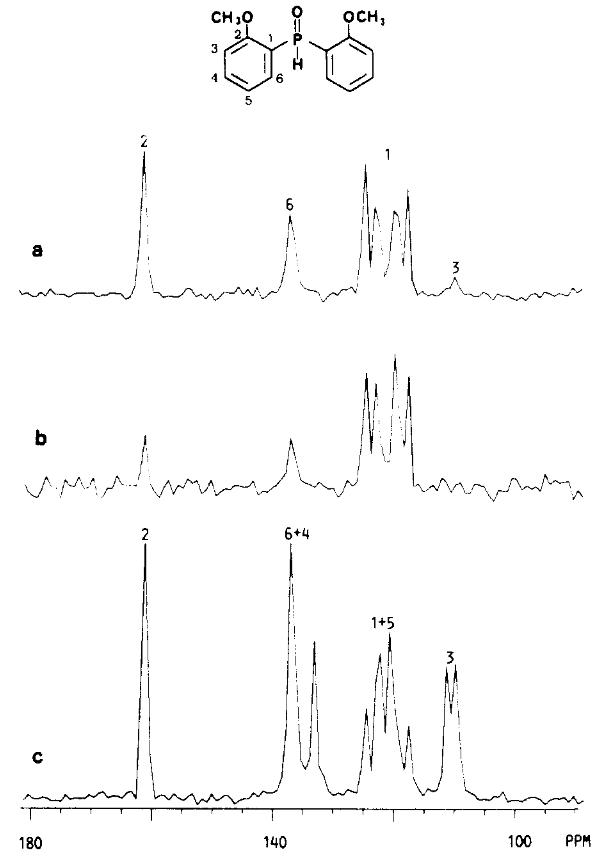

Figure 1. Conventional ^{1}H–^{13}C CP/MAS ^{13}C NMR spectrum (1c) and ^{1}H–^{13}C–^{31}P DCP/MAS ^{13}C NMR spectra (1a,b) of di-ortho-anisylphosphine oxide, **1**. All experiments used a 1 ms ^{1}H–^{13}C cross polarization contact time. DCP spectra 1a and 1b were generated with 15 and 1 ms ^{13}C–^{31}P cross polarization contact times, respectively.

On the basis of measurements of a limited, representative set of organophosphorus compounds, T_{CP} is a few ms for carbons directly bonded to phosphorus ($r < 0.2$ nm) and a few tens to hundreds of ms for carbons two- and three-bonds distant from the ^{31}P atom ($0.2 < r < 0.4$ nm). Typical ^{13}C $T_{1\rho}$ values for diamagnetic organic substances (30–200 ms) allow facile observation of carbons one-, two-, and three-bonds distant from the ^{31}P atom.[2] Schaefer et al. first reported the ^{1}H–^{13}C–^{15}N DCP/MAS NMR experiment as a method to identify carbons directly bonded to nitrogen and to estimate the concentration of ^{15}N–^{13}C bonds in a partially double-labeled solid.[1] The ^{1}H–^{13}C–^{31}P DCP/MAS NMR experiment differs from this seminal experiment by virtue of stronger dipolar coupling between the isolated cross polarization partners, facilitating the identification of carbons within a volume element that extends over several bond lengths.

Figures 1 and 2 illustrate the signal selection criteria of the ^{1}H–^{13}C–^{31}P DCP/MAS ^{13}C NMR experiment. Figure 1c displays the aromatic region of the conventional ^{1}H–^{13}C CP/MAS ^{13}C NMR spectrum of di-ortho anisyl phosphine oxide, **1**, with chemical shift assignments. Figure 1 (parts a and b) shows DCP spectra recorded with 15 and 1 ms ^{13}C–^{31}P cross polarization contact times, respectively. Each reveals resonances from those carbons one-bond removed, C(1), and two-bonds removed, C(2) and C(6), from the ^{31}P atom.[3] These are distinguished quantitatively by their T_{CP}, 2 and 16 ms, respectively, and qualitatively by comparison of signal intensities between the two DCP spectra. A low intensity peak from the resolved C(3), three-bonds distant

† This paper is dedicated to Clair J. Collins, an exemplary scientific scholar, by the author, protégé, and personal friend.

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(2) $T_{1\rho}$ values are rf field dependent. The range quoted here is from a collection of model measurements performed by using 45 KHz rf fields.

(3) Crystal lattice forces lift the symmetry plane appropriate for the rotationally averaged species in solution, making the aromatic rings inequivalent. The two resolved phosphorus-substituted carbon resonances each show $^{1}J_{CP}$ = 102 ± 6 Hz (solution value = 105 Hz).

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