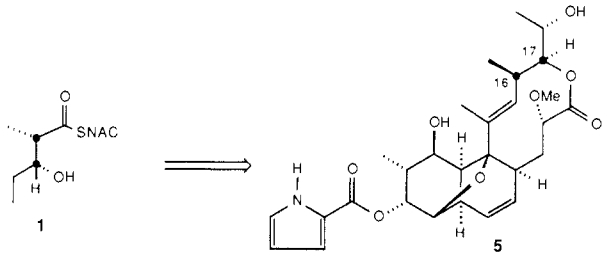
Scheme III



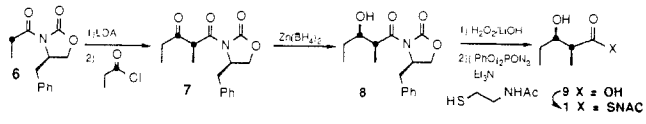
intermediate. Although nargenicin bears little structural resemblance to erythromycin, a consideration of the biosynthetic origins of each of these metabolites would indicate several close parallels in the elaboration of the parent polyketide chain. In order to probe these apparent biosynthetic similarities, we have investigated the incorporation of (2*S*,3*R*)-[2,3-$^{13}C_2$]-2-methyl-3-hydroxypentanoyl NAC-thioester (**1**)[6] into nargenicin.

Ten 70-mL fermentation cultures of *Nocardia agentinesis* Huang ATCC 31306 were incubated in 500-mL flasks at 30 °C and 250 rpm[5,8] for 24 h before administration of a total of 40 mg of [2,3-$^{13}C_2$]-**1** dissolved in 5 mL of 20% ethanol. Additional quantities of precursor were added after 48 h (20 mg) and 72 h (40 mg). After 96 h, the resulting crude nargenicin was extracted with ethyl acetate and purified by a combination of flash column chromatography and preparative TLC on silica gel. The 100.6 MHz ^{13}C NMR spectrum of the labeled nargenicin A_1 (7.1 mg) displayed the predicted set of enhanced and coupled doublets (J_{CC} = 36.2 Hz, 0.2 atom% enrichment), centered at 32.76 and 78.79 ppm, corresponding to C-16 and C-17, respectively.[5]

The observation of coupled ^{13}C NMR signals establishes the intact incorporation of the labeled thioester **1** into nargenicin, indicating that the polyketide synthetase of *N. argentinesis* can utilize a partially elaborated intermediate of the chain elongation process. These results are consistent with a chain elongation scheme involving adjustment of functionality and stereochemistry of the growing polyketide chain prior to each condensation reaction. The observed incorporation of the (2*S*,3*R*)-enantiomer of **1** is expected based on the previously determined absolute configuration of nargenicin.[5] Incorporation experiments involving more advanced intermediates of the chain-elongation process are in progress.

Acknowledgment. This work was supported by the National Institutes of Health, Grant GM 22172. The Bruker AM-400 used in this work was purchased with funds provided by the NIH Division of Research Resources, RR 02458. We would also like to thank Professor David A. Evans for helpful information regarding the preparation of **1**.

(6) The preparation of 1 was carried out by using the methods of Evans[7] for the erythroselective construction of aldol intermediates. Thus [2′-^{13}C]-(4*R*)-*N*-propionyl-4-benzyloxazolidinone (**6**), prepared as described[7] from (4*R*)-4-benzyloxazolidinone and [2-^{13}C]propionyl chloride,[3] was treated with lithium diisopropylamide and the resulting *Z* enolate was acylated with 0.9 equiv of [1-^{13}C]propionyl chloride. Reduction of the β-ketoimide **7** with 1.1 equiv of $Zn(BH_4)_2$ (Et_2O, 20 °C, 15 min) gave (2′*S*,3′*R*)-[2′,3′-$^{13}C_2$]-*N*-(2′-methyl-3′-hydroxypentanoyl)oxazolidinone (**8**) in 67% yield. Hydrolytic removal of the chiral auxilliary (3 equiv of LiOH, 12 equiv of 30% H_2O_2, THF, 20 °C, 15 min) gave the enantiomerically pure acid **9** (64% yield) which was converted to **1** as previously described.[3]



(7) (a) Evans, D. A. *Aldrichimica Acta* **1982**, *15*(2), 23. Evans, D. A.; Ennis, M. O.; Le, T.; Mandel, N.; Mandel, G. *J. Am. Chem. Soc.* **1984**, *106*, 1154. (c) Evans, D. A.; Takacs, J. M.; McGee, L. R.; Ennis, M. D.; Mathre, D. J.; Bartroli, J. *Pure Appl. Chem.* **1981**, *53*, 1109. (d) Evans, D. A.; Britton, T. C.; Ellman, J. A. *Tetrahedron Lett.* **1987**, *28*, 6141.

(8) Celmer, W. D.; Chmurny, G. N.; Moppett, C. E.; Ware, R. S.; Watts, P. C.; Whipple, E. B. *J. Am. Chem. Soc.* **1980**, *102*, 4203; Celmer, W. D.; Cullen, W. P.; Moppett, C. E.; Jefferson, M. T.; Huang, L. H.; Shibakawa, R.; Tone, J. U.S. Patent 4 148 883, 1979.

An Antibody-Catalyzed Claisen Rearrangement

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Received March 22, 1988

Considerable effort is being focused on the design of highly selective catalysts for use in chemistry and biology. Two well-established approaches have involved the introduction of catalytic groups into cavity-containing hosts, such as crown ethers and cyclodextrins, and the modification of enzyme specificity by site-directed mutagenesis. More recently, it was demonstrated that the binding affinity and specificity of antibodies[3-7] could be exploited in the generation of selective catalysts for acyl transfer and carbon–carbon bond-cleaving reactions.[4-7] Because antibodies can be generated to virtually any molecule of interest,[8] this approach may not only lead to the development of tailor-made catalysts but also provide an opportunity to test general notions of enzymatic catalysis. We report here the generation and characterization of a monoclonal antibody which catalyzes the Claisen rearrangement of chorismic acid to prephenic acid (Scheme I).

The thermal 3,3-sigmatropic rearrangement of chorismate to prephenate has been demonstrated to occur through an asymmetric chairlike transition in which the carbon–oxygen bond is substantially broken, while carbon–carbon bond formation has not occurred to any appreciable extent.[9-12] The entropy and enthalpy for the uncatalyzed reaction are 20.71 kcal/mol and −12.85 eu, respectively.[11] The unimolecular rearrangement is also catalyzed approximately 10^6-fold by the enzyme chorismate mutase at the branch point in the biosynthesis of aromatic amino acids in bacteria and plants.[11,13-15] Although the enzymatic reaction has also been demonstrated to proceed through a chairlike transition state,[16] the mechanism by which chorismate mutase accelerates the rearrangement is not fully understood. It has been argued that the rate of the enzyme-catalyzed rearrangement can be attributed to immobilization of the substrate in a conformation appropriate for rearrangement.[13] However, recent mechanistic studies of the enzyme-catalyzed reaction have been interpreted in favor of an intermediate which is covalently linked to the enzyme.[17] An

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(2) Igen, Inc., 1530 East Jefferson Ave., Rockville, MD 20852.
(3) Pollack, S. J.; Jacobs, J. W.; Schultz, P. G. *Science (Washington, D. C.)* **1986**, *234*, 1570. Pollack, S. J.; Schultz, P. G. *Cold Spring Harbor Symposia Quantum Biology*; Cold Springs Harbor: New York, 1987; Vol. 52, in press.
(4) Jacobs, J.; Schultz, P. G.; Sugasawara, R.; Powell, M. *J. Am. Chem. Soc.* **1987**, *109*, 2174.
(5) Schultz, P. G. *Science (Washington, D. C.)* **1988**, *240*, 426.
(6) Tramontano, A.; Janda, K. D.; Lerner, R. A. *Science (Washington, D. C.)* **1986**, *234*, 1566.
(7) Napper, A. D.; Benkovic, S. J.; Tramontano, A.; Lerner, R. A. *Science (Washington, D. C.)* **1987**, *237*, 1041.
(8) Pressman, D.; Grossberg, A. *The Structural Basis of Antibody Specificity*; Benjamin: New York, 1968.
(9) Copley, S. D.; Knowles, J. R. *J. Am. Chem. Soc.* **1985**, *107*, 5306. Addadi, K.; Jaffe, E. K.; Knowles, J. R. *Biochemistry* **1983**, *22*, 4494.
(10) Coates, R. M.; Rogers, B. D.; Hobbs, S. J.; Peck, D. R.; Curran, D. P. *J. Am. Chem. Soc.* **1987**, *109*, 1160.
(11) Andrews, P. R.; Smith, G. D.; Young, I. G. *Biochemistry* **1973**, *12*, 3492.
(12) Gajewski, J. J.; Jurayi, J.; Kimbrough, D.; Gande, M. E.; Ganem, B.; Carpenter, B. K. *J. Am. Chem. Soc.* **1987**, *109*, 1170.
(13) Gorisch, H. *Biochemistry* **1978**, *17*, 3700.
(14) Halsam, E. *The Shikimate Pathway*; Wiley: New York, 1974; Ganem, B. *Tetrahedron* **1978**, *34*, 3353.
(15) Koch, G. L.; Shaw, D. C.; Gibson, F. *Biochim. Biophy. Acta* **1972**, *258*, 719.
(16) Sogo, S. G.; Widlanski, T. S.; Hoare, J. H.; Grimshaw, C. E.; Berchtold, G. A.; Knowles, J. R. *J. Am. Chem. Soc.* **1984**, *106*, 2701.

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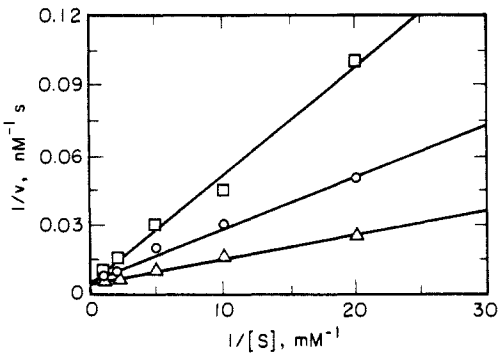
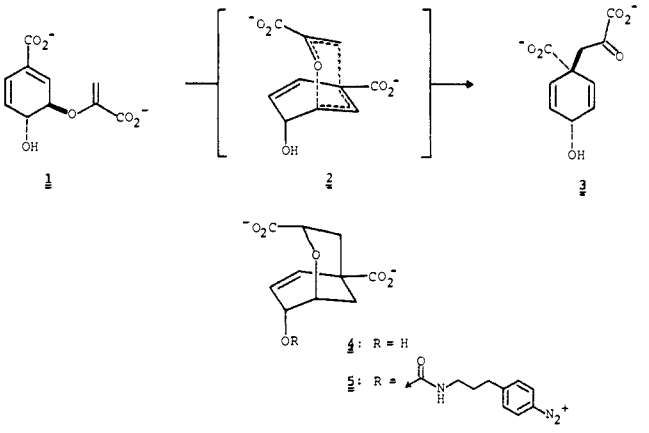


Figure 1. Lineweaver–Burk plot for antibody-catalyzed Claisen rearrangement. Velocities were determined by measuring the initial linear absorbance at 270 nm: △, no inhibitor present; ○, inhibited by 10 μM **4**; □, inhibited by 20 μM **4**.

Scheme I



intriguing question then arises—to what extent can an antibody combining site, complementary to the conformationally restricted transition state configuration but presumably lacking catalytic side chains, accelerate the Claisen rearrangement of chorismate to prephenate?

In order to test this notion, monoclonal antibodies were elicited to the endo bicyclic transition-state analogue **4**. Analogue **4**, in which the two carboxyl groups are positioned to mimic the chairlike transition-state configuration **2**, is the most potent known inhibitor of chorismate mutase with a K_i of 0.15 μM.[18,19] Compound **4** was converted to the carbamate–diazonium derivative **5** and conjugated to the carrier proteins keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA).[20] Epitope densities were determined based on A_{370} and ranged from 5 to 12 per carrier. Monoclonal antibodies specific for hapten **5** were obtained by standard protocols[4,21] and purified to homogeneity (as judged by SDS gel electrophoresis[22]) by chromatography on protein A-coupled Sepharose 4B.[23]

The rate of the rearrangement of chorismate to prephenate was assayed spectrophotometrically (270 nm) at pH 7.0 (5 mM NaCl, 50 mM Na_2PO_4 buffer) in the absence (k_{un}) and presence of antibody (k_{obsd}); the reactions were followed at 10 °C to minimize subsequent decomposition of prephenate.[24] One of eight antibodies (IgG) assayed was found to catalyze the Claisen rearrangement, with initial rates consistent with the Michaelis–Menten rate expression 1. The values of k_{cat} and K_m are 2.7 min^{-1} and

$$\text{Ig} + \mathbf{1} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Ig}\cdot\mathbf{1} \xrightarrow{k_{cat}} \text{Ig} + \mathbf{3} \qquad (1)$$

260 μM, respectively, at 10 °C. High pressure liquid chromatographic (HPLC) analysis confirmed that the sole product (>99%) of the antibody-catalyzed reaction is prephenic acid.[25] The uncatalyzed reaction afforded prephenate and *p*-hydroxybenzoate in a molar ratio of 19:1.[25] There is no solvent isotope effect on the antibody-catalyzed rearrangement in D_2O. The racemic endo diacid **4** inhibits the antibody-catalyzed reaction competitively with a K_i of 9 μM at 10 °C. The dimethyl ester of chorismic acid is not a substrate for the antibody,[26] indicating that the carboxylate groups play an important role in antibody-ligand recognition. However, the antibody does catalyze the rearrangement of the (±)-methyl ether of chorismic acid to the prephenate methyl ether with values of k_{cat} and K_m of 0.87 min^{-1} and 480 μM, respectively.[24,25]

The rate of the antibody-catalyzed reaction can be directly compared with that of the uncatalyzed thermal rearrangement affording a value of k_{cat}/k_{un} of 1×10^4 at 10 °C, pH 7.0. This factor can be contrasted with the 3×10^6-fold acceleration induced by chorismate mutase from *E. coli* under the same conditions.[27] Of the various mechanisms put forth for the enzymatic rearrangement, most can be ruled out for the antibody-catalyzed process. For example, the fact that the (±)-methyl ether of chorismate is retained in the transformation argues against mechanisms involving loss of the 4-hydroxyl group (such as formation of an oxirinium ion or C-4 cation). Moreover, the observation of a D_2O solvent isotope effect of unity on the antibody-catalyzed reaction rules out general acid or base catalysis in the rate-limiting step. One is left with the appealing mechanistic alternative that the antibody catalyzes the reaction by providing an environment complementary to the conformationally restricted transition state. Additional experiments are being pursued to more precisely define the origin of the rate acceleration. Finally, this work represents the extension of antibody catalysis to another class of reactions and is the first report of antibody-catalyzed carbon–carbon bond formation.[30]

Supplementary Material Available: Full experimental details for the synthesis and characterization of **5**, protein conjugation, preparation and purification of substrates and products, and isolation of antibodies (4 pages). Ordering information is given on any current masthead page.

(17) Guilford, W. J.; Copley, S. D.; Knowles, J. R. *J. Am. Chem. Soc.* **1987**, *109*, 5013.
(18) Bartlett, P. A.; Nakagawa, Y.; Johnson, C. R.; Reich, S. H.; Luis, A. *J. Org. Chem.* **1988**, in press.
(19) Bartlett, P. A.; Johnson, C. R. *J. Am. Chem. Soc.* **1985**, *107*, 7792.
(20) Reaction of 4-(4-nitrophenyl)butyric acid with diphenylphosphoryl-azide and triethylamine in benzene at reflux, followed by addition of the dimethyl ester of **4** and continued heating afforded the nitrophenyl derivative of **4**.[28] Reduction of the nitro group (H_2, PtO_2), saponification of the methyl esters (NaOH, H_2O–MeOH), and treatment with $NaNO_2$ under acidic conditions afforded the dicarboxylate, diazonium salt **5**. The diazonium salt was coupled to BSA and KLH under basic conditions at 0 °C,[29] and the resulting conjugate dialyzed against 150 mM NaCl, 10 mM Na_2PO_4, pH 7.4 buffer.
(21) Sugasawara, R.; Prato, C.; Sippel, J. *Infect. Immun.* **1983**, *42*, 863.
(22) Laemmli, V. *Nature (London)* **1970**, *227*, 680.
(23) Kronvall, G.; Grey, H.; Williams, R. J. *Immunol.* **1970**, *105*, 1116.
(24) A stock solution of chorismic acid in 5 mM NaCl, 50 mM sodium phosphate, pH 7.0 buffer, was added (1:100 dilution) to a solution of antibody in the same buffer at 10 °C to give a final antibody concentration of 5 μM ($\epsilon = 1.37^{0.1\%}_{1\,cm}$ with a molecular weight of 150 000 for immunoglobulin G) and substrate concentrations between 1 and 1000 μM. The first-order rate constant (k_{uncat}) for the Claisen rearrangement in the absence of antibody was measured similarly and used to correct the initial rate data.
(25) Products were analyzed by reverse phase HPLC (Bondapak C_{18}, 5% CH_3CN in 0.1 M aqueous triethylammonium acetate buffer, pH 5).
(26) Ife, R. J.; Ball, L. F.; Lowe, P.; Haslam, E. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1776.
(27) The k_{cat} and K_m of chorismate mutase (*E. coli*) were determined to be 13.6 s^{-1} and 290 mM, respectively, at 10 °C in 5 mM NaCl, 50 mM Na_2PO_4, pH 7.0[24] (note, these are different assay conditions than those in ref 19).
(28) Yamada, S.; Ninomiya, K.; Shioiri, T. *Tetrahedron Lett.* **1973**, 2343.
(29) Thomas, C. *Experimental Immunochemistry*, 2nd ed.; Springfield: IL, 1961.
(30) An antibody that catalyzes the Claisen rearrangement of chorismic acid at a reduced rate has been independently generated by other investigators: Hilvert, D., et al. *Proc. Nat. Acad. Sci. U.S.A.* **1988**, in press.
(31) We acknowledge the gift of methyl ether of chorismic acid from Professor Glenn Berchtold (M.I.T.) and the gift of chorismate mutase from Professor Jeremy Knowles (Harvard University). This work was supported by grants from the National Institutes of Health (Grant AI24695-02 to P.G.S. and GM-28965 to P.A.B.).