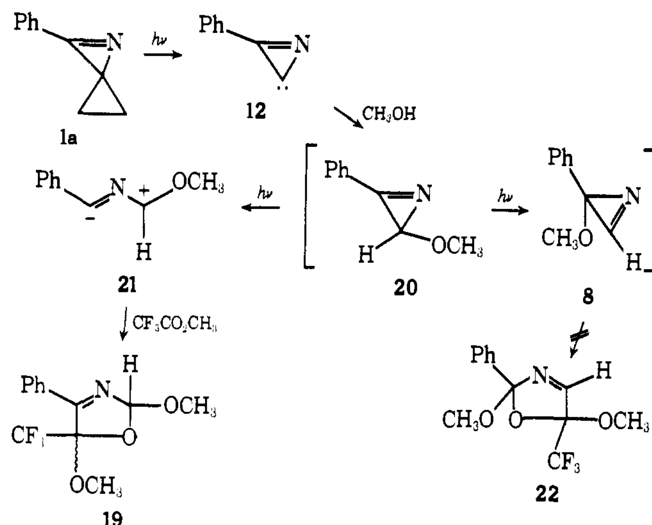


Acknowledgment. We gratefully acknowledge support of this work by the National Science Foundation (Grant PO-37550) and the National Institutes of Health (Grant No. CA-12195-09).

References and Notes

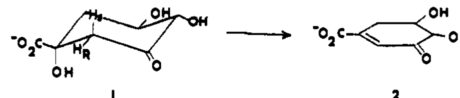
(1) Photochemical Transformations of Small Ring Heterocyclic Compounds. 69. For part 68 see A. Padwa and D. Dehm, *J. Am. Chem. Soc.*, **97**, 4779 (1975).
(2) A. Padwa and J. Smolanoff, *J. Am. Chem. Soc.*, **93**, 548 (1971).
(3) A. Padwa, S. Clough, M. Dharan, J. Smolanoff, and S. I. Wetmore, Jr., *J. Am. Chem. Soc.*, **94**, 1395 (1972); **95**, 1945, 1954 (1973).
(4) A. Padwa, D. Dean, and J. Smolanoff, *Tetrahedron Lett.*, 4087 (1972).
(5) A. Padwa, J. Smolanoff, and S. I. Wetmore, Jr., *J. Org. Chem.*, **38**, 1333 (1973).
(6) A. Padwa and S. I. Wetmore, Jr., *J. Am. Chem. Soc.*, **96**, 2414 (1974).
(7) N. Gakis, M. Marky, H. J. Hansen, and H. Schmid, *Helv. Chim. Acta*, **55**, 748 (1972).
(8) H. Giezendanner, M. Marky, B. Jackson, H. J. Hansen, and H. Schmid, *Helv. Chim. Acta*, **55**, 745 (1972).
(9) B. Jackson, M. Marky, H. J. Hansen, and H. Schmid, *Helv. Chim. Acta*, **55**, 919 (1972).
(10) A. Padwa and J. Smolanoff, *J. Chem. Soc., Chem. Commun.*, 342 (1973).
(11) P. Claus, P. Gilgen, H. J. Hansen, H. Heimgartner, B. Jackson, and H. Schmid, *Helv. Chim. Acta*, **57**, 2173 (1974).
(12) Azirines 1, were prepared according to literature procedures, or by modifications of those procedures, H. J. Bestmann and R. Kunstmann, *Chem. Ber.*, **102**, 1816 (1969); S. Sato, *Bull. Chem. Soc. Jpn.*, **41**, 1440 (1968).
(13) The spectral properties of all new compounds and the full experimental details will be presented in our full publication. The yield reported for the formation of 3-phenyl-3-methoxyazirine (**8**) was determined from a short irradiation experiment. Also, the total yield of **9** and **10** amounted to 8%.
(14) G. W. Griffin and N. R. Bertoniere in "Carbenes", Vol. 1, M. Jones and R. A. Moss, Ed., Wiley, New York, N. Y., 1973.
(15) W. Bauer and K. Hafner, *Angew. Chem., Int. Ed. Engl.*, **8**, 772 (1969).
(16) W. M. Jones, M. E. Stowe, E. E. Wells, Jr., and E. W. Lester, *J. Am. Chem. Soc.*, **90**, 1849 (1968), and references cited therein.
(17) W. Kirmse, L. Horner, and H. Hoffmann, *Justus Liebigs Ann. Chem.*, **614**, 19 (1958).
(18) A. Hassner and R. J. Isbister, *Tetrahedron*, **25**, 1637 (1969).
(19) Irradiation of **1a** in the presence of excess piperylene under nitrogen produced no significant increase in the yield of benzonitrile (ca. 4%), thus eliminating the possibility that oxygen was acting only as a triplet quencher. Experiments are underway to spectroscopically detect azirinone **13** at low temperatures.
(20) Control experiments established that **6** was stable toward irradiation. This observation eliminates a Griffin fragmentation of **6** to an iminocarbene followed by reaction with methanol as the path responsible for the formation of methoxyimine **11**.
(21) W. Sieber, P. Gilger, S. Chaloupka, H. J. Hansen, and H. Schmid, *Helv. Chim. Acta*, **56**, 1679 (1973).
(22) It is conceivable that cycloadduct **22** is formed initially but rearranges to **19** under the reaction conditions. However, all attempts to detect **22** in the crude photolysate have failed thus ruling out this possibility.
(23) It should be pointed out that **8** was the only azirine detected in the crude photolysate. An alternate explanation to account for its formation is that 2-phenylazirinylidene (**12**) reacts with methanol to give both **8** and **20**. Under the photolytic conditions (Corex filter), the more strongly absorbing azirine (i.e., **20**) undergoes subsequent secondary photochemistry (e.g., formation of **11**) and leaves behind the weaker absorbing isomer (i.e., **8**). Attempts to synthesize **20** in order to verify this point are in progress.
(24) J. Ciabattoni and M. Cabell, Jr., *J. Am. Chem. Soc.*, **93**, 1482 (1971).
(25) National Institutes of Health Postdoctoral Fellow, 1974–1975.

Albert Padwa,* Jerald K. Rasmussen[25]
Department of Chemistry
State University of New York at Buffalo
Buffalo, New York 14214
Received May 15, 1975

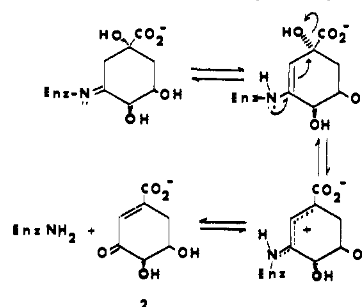
Dehydroquinase Catalyzed Dehydration. II. Identification of the Reactive Conformation of the Substrate Responsible for Syn Elimination[1]

Sir:

Beginning with the demonstration of the syn dehydration catalyzed by the enzyme dehydroquinase,[2] this catalytic mechanism, as an example of rarely observed biological syn dehydration, has attracted much speculation.[3,4] Although overshadowed by the stereospecific pro-*R* proton abstraction of the enzymic reaction, the stereoselective pro-*S* proton abstraction of the base-catalyzed enolization of dehydroquinate (**1**)[5] is an important key in understanding the stereochemistry of the enzymic reaction. The enzymic conversion of **1** to dehydroshikimate (**2**) involves a Schiff base



intermediate.[1] Thus, the mechanistic sources of the anti stereochemistry, which are observed in eliminations involving both enolates and Schiff base intermediates,[6] must be circumvented in the parallel mechanistic conversion of the enzyme Schiff base to the enzyme enamine. It is this step which determines the syn stereochemistry of the biological elimination. Chemical modification of both the carboxyl function at C-1 and the hydroxyl functions at C-4 and C-5 in the substrate **1** have allowed the identification of the mechanistic source of this unusual syn dehydration.



We have synthesized the methyl ester of **1** by treatment of the silver salt derived from **1** with methyl iodide. The compound is obtained as an oil; after ion exchange chromatography, its NMR spectrum is superposable with that of **1** except for the methyl resonance at 4.31 ppm.[7] This ester is neither a substrate nor an inhibitor for dehydroquinase; thus the carboxyl group of **1** is an important site for binding the substrate to the enzyme. Haslam and coworkers have suggested that the carboxylate, acting as an internal base, is responsible for the pro-*S* stereoselectivity of the nonenzymatic enolization of **1**.[5] To the extent that such a mechanism is important, binding the carboxylate to the enzyme could negate this pro-*S* stereoselectivity of the enolization process and thus accentuate other factors which could control the stereochemistry of the biological elimination.[6] We have tested this hypothesis by comparing the pseudo-first-order

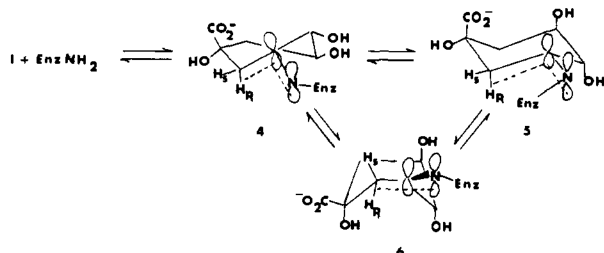
Table I. Rates of Deuterium Incorporation from D_2O in Sodium Benzoate 0.237 *M*, pH 7.0 at 34.40°, Ionic Strength = 1.44

Compound	$k_S{}^a$ (hr^{-1})	$k_S/k_r{}^b$
1	$(2.12 \pm 0.06) \times 10^{-3}$	6.65
Methyl ester of 1	$(2.05 \pm 0.07) \times 10^{-3}$	6.70

[a] Pseudo-first-order rate constant for deuterium incorporation in the pro-*S* position at C-2. [b] Relative rate of incorporation of deuterium into pro-*S* and pro-*R* positions.

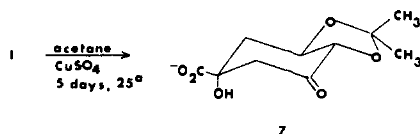
rates of deuterium incorporation from D_2O at C-2 from **1** and its methyl ester. These results are shown in Table I. Clearly, masking the carboxylate function with a methyl group changes neither the stereochemistry nor the rate of enolization. Thus, binding of the carboxylate to the enzyme, while important, cannot explain the reversed stereochemistry of the biological elimination. The pro-*S* stereoselectivity of the nonenzymatic enolization and the resulting anti elimination must then derive from the stereoelectronic necessity for overlap between the n orbitals of the carbonyl oxygen of **1** and the σ orbitals of the adjacent axial proton.[8] In the most stable conformation of **1**[9] only the pro-*S* proton meets this axial requirement. Since a Schiff-base is the electronic counterpart of a ketone, these same considerations would predict that the enzyme Schiff-base substrate complex would also undergo anti elimination from a conformation of the substrate such as **1**. That a syn elimination occurs strongly implies that the substrate undergoes a conformational change during the enzyme catalyzed process.

There are three distinct conformational changes that can occur, **4**, **5**, and **6**, any one of which will lead to the required



overlap. Skew-boat conformation **4** differs from **5** and **6** in important ways; namely, the OH groups remain diequatorial, and this conformer can be generated from enzyme and **1** by movement of only the carboxyl-bearing carbon and those carbons adjacent to it. These distinguishing features allow an experimental test for the reactive substrate conformation.

We have synthesized the isopropylidene derivative (**7**) from **1** by the route shown below. The ketal (**7**) was obtained as a crystalline solid, mp 124–127, from ethyl acetate.[7] The superposability (except for the methyl resonances at 2.04 and 2.20 ppm) of the NMR spectrum of **7** with that of **1** shows the identity of the ring hydrogen coupling constants in both compounds and assures that the cyclohexane ring of **7** has a conformation which is identical with **1**.[9]



Models show that **7** unlike **1** cannot assume the conformations represented by **5** and **6** since the diequatorial hydroxyl groups at C-4 and C-5 are locked by the fused five-membered ketal ring.

Ketal **7** at 0.3 *M* is a reactive substrate for dehydroquinase and reacts with the enzyme at a rate 0.553 that of the natural substrate **1** in 0.033 *M* Tris at pH 7.4. That prior hydrolysis of **7** to **1** does not occur under the reaction conditions is shown by the fact that treatment of **7** with a solution of the enzyme at pH 7.8 for 12–13 hr produces more than 99% of the elimination product with the fused ketal ring intact; only a trace of the hydrolysis product can be detected by NMR at this pH. The rate of elimination of **7** is slower than that of the natural substrate **1**, and an equimolar mixture of **1** and **7**, each at 0.3 *M*, reacts only 0.714 times as fast as **1**, i.e., **7** inhibits the enzymatic elimination of **1**. Since a conformational change is required in this reaction and since the only conformational change available to **7** is that corresponding to **4**, we propose that the OH groups at C-4 and C-5 remain diequatorial throughout this reaction.

While this work was in progress, Haslam and coworkers proposed that the reactive conformation of dehydroquinate was the skew-boat **6**.[4] Since this conformation has diaxial OH groups at C-4 and C-5, it cannot be the reactive conformation in light of the present evidence. That the carboxyl group at C-1 should become more nearly axial during this conformational change is in complete accord with the evidence that this carboxylate is necessary for substrate reactivity. We believe that it is this carboxylate group which together with the ketone carbonyl engages the enzyme and sets into play the conformational change to **4** which results in the syn elimination. Such a conformational change in the substrate may be necessary to produce a corresponding conformational change in the enzyme which is necessary to align the proton abstracting base on the enzyme or any other groups necessary for the reaction to proceed. We expect to comment on these features in a future publication.

Acknowledgment. This work was supported by a Biomedical Support Grant to Tulane University. We are grateful to W. L. Alworth for much helpful discussion and to James Leming for technical assistance.

References and Notes

(1) For the first paper in this series see J. R. Butler, W. L. Alworth, and M. J. Nugent, *J. Am. Chem. Soc.*, **96**, 1617 (1974).
(2) K. R. Hanson and I. A. Rosè, *Proc. Nat. Acad. Sci. U.S.A.*, **50**, 981 (1963).
(3) I. A. Rose, *Crit. Rev. Biochem.*, Feb., 35–57, (1972); I. A. Rose, "The Enzymes", Vol. II, 3rd ed, P. D. Boyer, Ed, Academic Press, N.Y., 1970, p 309; K. R. Hanson and I. A. Rose, *Acc. Chem. Res.*, **8**, 1 (1975).
(4) M. J. Turner, B. W. Smith, and E. Haslam, *J. Chem. Soc., Perkin Trans. 1*, 52 (1975).
(5) E. Haslam, M. J. Turner, D. Sargent, and R. S. Thompson, *J. Chem. Soc. C*, 1489 (1971).
(6) That elimination proceeds through the enolate or Schiff base intermediates in the nonenzymic elimination of β-hydroxyketols has been shown by D. J. Hupe, M. C. R. Kendall, G. T. Sinner, and T. A. Spencer, *J. Am. Chem. Soc.* **95**, 2260 (1973); D. J. Hupe, M. C. R. Kendall, and T. A. Spencer, *ibid.*, **95**, 2271 (1973).
(7) Satisfactory C, H analysis and infrared and NMR spectra were obtained for this compound.
(8) For a review of this effect see H. O. House, "Modern Synthetic Reactions", 2nd ed, W. A. Benjamin, Menlo Park, Calif., 1972, pp 499–500.
(9) J. Corse, R. E. Lundin, E. Sondheimer, and A. C. Waiss, Jr., *Phytochemistry*, **5**, 767 (1966).
(10) The rate of reaction of substrate 1 and of isopropylidene derivative 7 were monitored by the increase in absorbance at 234 nm.

Alfin D. N. Vaz, James R. Butler, Maurice J. Nugent*
Laboratory of Chemical Biology
Department of Chemistry, Tulane University
New Orleans, Louisiana 70118
Received May 30, 1975

Metal Hydroxide Promoted Hydrolysis of Carbonyl Substrates

Sir:

The recently reported hydration of CO_2 by "inert" metal hydroxides of the type $(NH_3)_5MOH^{2+}$ (M = Co,[1] Rh,[2] Ir[2])