The sensitivity of hydride **3** frustrated attempts to study this reaction kinetically. However, rearrangement of the somewhat better behaved iodides gave reproducible rate data, and these provided support for a ring-opening mechanism involving polar zwitterionic intermediates such as **B**. The rate of conversion of **5a** to **6a** was measured by UV-vis spectroscopy. The rearrangement is first order in [**5a**] and is accelerated by polar solvents (rate constrants at 20 °C in toluene, THF, and acetone, respectively: 2.1, 4.4, and 10×10^{-4} s^{-1}; estimated error ±10%). In toluene solution, the reaction has a large negative entropy of activation,[19] further supporting the intervention of a charge-separated transition state.[20]

In summary, our results demonstrate that epoxides react similarly to cyclopropanes[11,21] with the intermediate generated photochemically from dihydride **1** by initial oxidative addition of rhodium into a three-membered ring C-H bond. Once metalated, however, the small ring compounds lead to different final products: the cyclopropylrhodium compound gives metallacyclobutane,[21] while the expoxyrhodium complex rearranges to an enolate, apparently by a hydrogen-shift rather than a rhodium-shift mechanism.[22] Whatever the precise mechanism of the **3a** → **4a** and **5a** → **6a** rearrangements, however, the experiments described here demonstrate that at least in some instances, overall metal-induced ring-opening reactions of small heterocyclic organic compounds need not begin by cleavage of the ring.

Acknowledgment. This work was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Chemical Sciences Division of the United States Department of Energy under contract No. DE-AC03-76SF00098. We are grateful to Prof. Bruce Rickborn (University of California, Santa Barbara) for helpful discussions regarding rearrangements of alkali-metalated epoxides. We acknowledge Dr. F. J. Hollander, Director of the U. C. Berkeley College of Chemistry X-ray Diffraction Facility, for carrying out the X-ray structure determination, and we also appreciate a generaous gift of rhodium chloride from the Johnson-Matthey Co. Metal Loan program.

Supplementary Material Available: Spectroscopic data supporting the structural assignments of **3a**, **4a**, **5a**, and **6a** and full details of the X-ray diffraction study, including ORTEP stereodrawings and tables of crystal and data collection parameters, positional parameters, anisotropic thermal parameters, intramolecular distances and angles, and least-squares planes (15 pages); table of calculated and observed structure factors (15 pages). Ordering information is given on any current masthead page.

(17) A referee has asked us to mention that C-H oxidative addition was suggested in an early paper (ref 8) as the initial step in a rhodium-catalyzed rearrangement of stilbene oxides to deoxybenzoins at high temperature (150-210 °C). However, the second step postulated in the previous mechanism involved 1,3-transfer of rhodium-bound hydride to the epoxide carbon atom to open the ring, followed by extrusion of the metal. This mechanism cannot be operating in the **3a** to **4a** rearrangement, because it would lead to products that are not observed (an acyl hydride or acetaldehyde), nor can it be operating in the rearrangement of iodide **5a** to **6a**, since in this case iodide, rather than hydride, is attached to rhodium. We suggest that a hydrogen-migration mechanism analogous to the one described here may also operate in the stilbene oxide-to-deoxybenzoin rearrangement.

(18) Interestingly, a similar mechanism has been postulated to occur through a carbenoid species in the rearrangement of alkali-metalated epoxides to enolates; cf.: (a) Thummel, R. P.; Rickborn, B. *J. Org. Chem.* **1972**, *37*, 3919. (b) Crandall, J. K.; Apparu, M. *Org. React.* **1983**, *29*, 345, and references cited therein.

(19) Activation parameters in toluene solvent were determined by measuring rate constants over the temperature range from 0-35 °C: $\Delta H^{\ddagger} = 12.0$ Kcal/mol; $\Delta S^{\ddagger} = -34.5$ eu.

(20) Chock, P. B.; Halpern, J. *J. Am. Chem. Soc.* **1966**, *88*, 3511.

(21) Periana, R. A.; Bergman, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 7346.

(22) A referee has inquired about the products formed on irradiation of $Cp^*(L)RhH_2$ with methyl-substituted epoxides. We have briefly investigated the reaction with 2,2-dimethyloxirane; observation by low-temperature NMR indicates that insertion occurs into both the methyl and ring C-H bonds (as it does with cyclopropane), but warming leads to a complex mixture of products. Because of this and the fact that photochemical conversions were lower than with ethylene oxide itself, this reaction has not been investigated further.

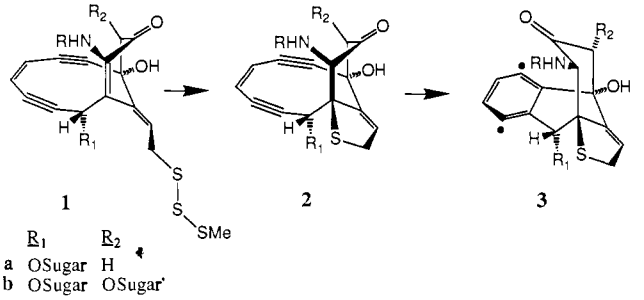
The Cyclization of Calicheamicin-Esperamicin Analogues: A Predictive Biradicaloid Transition State

James P. Snyder

Drug Design Section, G. D. Searle Research and Development, 4901 Searle Parkway Skokie, Illinois 60077

Received May 4, 1989

A family of exceptionally potent antitumor agents were recently isolated from soils found in Texas and Argentina. Elegant structural and mechanistic studies by Lederle[1] and Bristol-Myers/Cornell[2] teams have characterized the active agents as the highly unsaturated bicyclics calicheamicin γ_1 (**1a**) and esperamicin A_2 (**1b**), respectively. Biological action is intimately linked to double-stranded DNA cleavage.[3,4] Nicolaou and co-workers have prepared a highly simplified monocyclic analogue of **1** (**4**, Z = $(CHCH_2OH)_2$) and shown it both to cyclize in benzene and to cause scission of a variant of DNA at 37 °C.[5] Other total synthetic efforts are likewise underway.[6] The current mechanistic hypothesis[1-3] is that **1** binds to the minor groove of the double strand, undergoes Michael addition to give **2**, and finally experiences spontaneous Bergman ring closure[7] to the fugitive but lethal biradical **3**. The latter is thought to abstract hydrogen from the DNA polymer to produce oligonucleotide fragments and the corresponding benzannelated structures as intermediate metabolites.



In an attempt to define the pathway from **4** to **7**, the present work describes a quantum mechanical model for cyclization to the biradicaloid transition state **5**. Two important questions concern (1) the degree of biradical character in **6** and (2) whether the pathway is least motion or not. The transformation of several

(1) Lee, M. D.; Dunne, T. S.; Siegel, M. M.; Chang, C. C.; Morton, G. O.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3464-3466. Lee, M. D.; Dunne, T. S.; Chang, C. C.; Ellestad, G. A.; Siegel, M. M.; Morton, G. O.; McGahren, W. J.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3466-3468.

(2) Golik, J.; Clardy, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Kirshnan, B.; Ohkuma, H.; Saitoh, K.-i.; Doyle, T. W. *J. Am. Chem. Soc.* **1987**, *109*, 3461-3462. Golik, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.-i.; Doyle, T. W. *J. Am. Chem. Soc.* **1987**, *109*, 3462-3464.

(3) Zein, N.; Poncin, M.; Nilakantan, R.; Ellestad, G. A. *Science* **1989**, *244*, 697-699. Zein, N.; Sinha, A. M.; McGahren, W. J.; Ellestad, G. A. *Science* **1988**, *240*, 1198-1201. Hawley, R. C.; Kiessling, L. L.; Schreiber, S. L. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1105-1109.

(4) Neocarzinosatin chromophore, a related antitumor antibiotic, likewise cleaves DNA upon aerobic incubation: Kappen, L. S.; Ellenberger, T. E.; Goldberg, I. H. *Biochemistry* **1987**, *26*, 384-390. Myers, A. G.; Proteau, P. J.; Handel, T. M. *J. Am. Chem. Soc.* **1988**, 7212-7214.

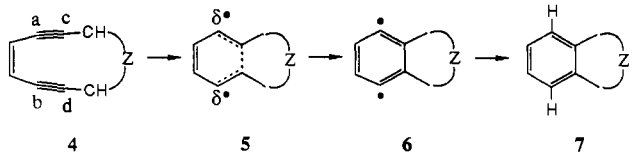
(5) Nicolaou, K. C.; Ogawa, Y.; Zuccarello, G.; Kataoka, H. *J. Am. Chem. Soc.* **1988**, *110*, 7247-7248.

(6) (a) Carter, P. A.; Magnus, P. *J. Am. Chem. Soc.* **1988**, *110*, 1626-1628. Magnus, P.; Lewis, R. T.; Huffman, J. C. *Ibid.* **1988**, *110*, 6921-6923. (b) Danishefsky, S. J.; Mantlo, N. B.; Yamashita, D. S. *J. Am. Chem. Soc.* **1988**, *110*, 6890-6891. Danishefsky, S. J.; Yamashita, D. S.; Mantlo, N. B. *Tetrahedron Lett.* **1988**, 4681-4684. (c) Schreiber, S. L.; Kiessling, L. L. *J. Am. Chem. Soc.* **1988**, *110*, 631-633. *Tetrahedron Lett.* **1989**, 433-436. (d) Kende, A. S.; Smith, C. A. *Tetrahedron Lett.* **1988**, 4217-4220. (e) Tomioka, K.; Fujita, H.; Koga, K. *Tetrahedron Lett.* **1989**, 851-854.

(7) (a) Lockhart, T. P.; Comits, P. B.; Bergman, R. G. *J. Am. Chem. Soc.* **1981**, *103*, 4082-4090. (b) Bergman, R. G. *Acc. Chem. Res.* **1973**, *6*, 25-31.

0002-7863/89/1511-7630$01.50/0

enediynes is examined in this context.

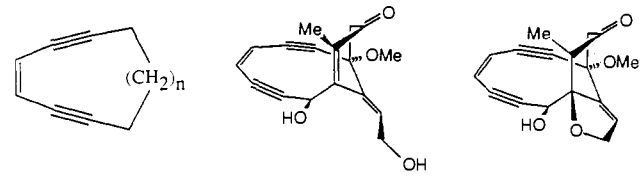


4 5 6 7

Ring closure of **4** to 1,4-diyl **6** at minimum is a four-electron problem covering fusion of the in-plane acetylene π bonds. Simultaneously six π electrons form an aromatic benzene ring. To accommodate the ten-electron situation a PRDDO-GVB-CI treatment[8] over selected hybrid localized MO's[9] was employed. This machinery was applied in the framework of a linear synchronous transit pathway between 4-octene-2,6-diyne (**4**, Z = H H) and 1,4-*o*-xylenediyl (**6**, Z = H H).[10] The geometry optimized planar transition state is depicted in Figure 1.[11] Nonplanar geometries involving torsions about the CH—C≡C bonds prove to be of higher energy. The result strongly suggests a least motion closure pathway and reveals 35% biradical character[12] in transition state **5**.

To provide transition-state structures for a range of substitution types, the MM2 force field[13] was parameterized to reproduce the PRDDO-GVB-CI transition state (Figure 1). Enediyne ground-state geometries were evaluated in the same framework with "soft" C—C≡C bending force constants.[14] Relative transition-state energies were then obtained by fixed-point PRDDO calculations: ΔE^* = **4**(PRDDO-SCF) − **5**(PRDDO-GVB).

As a first application of the 1,4-diyne reaction model, monocyclic series **8** (n = 1–3) was examined. The 10- and 11-membered



8 9 10

rings (n = 2,3) required a conformational ring search prior to PRDDO energy evaluation. Calculated ΔE^*'s for the structures are 17.8, 24.7 (E_{act} = 23.8[15]), and 35.9 kcal/mol corresponding to low-energy diyne conformations with r_{cd} = 2.99, 3.46, and 3.78 A (cf. **4**), respectively. Previously an MM2 calculated value of r_{cd} < 3.3 A was proposed as signifying spontaneous ambient closure.[15] The energetic predictions are in complete qualitative and quantitative accord with spontaneous cyclization of **8** (n = 1), the experimental barrier of **8** (n = 2), and the indefinite ambient stability of **8** (n = 3). The correspondence supports the cd distance criterion as reflecting movement along a least motion closure pathway in the monocyclic series induced synthetically by the incorporation of increased enediyne ring strain. The r_{cd}

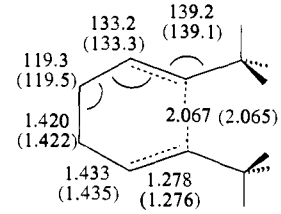
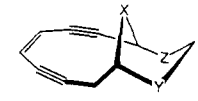


Figure 1. PRDDO-GVB-CI transition state for the Bergman cyclization of octa-2,6-diyn-4-ene. The MM2 optimized values are in parentheses; bond angles above (deg), bond lengths below (Å).

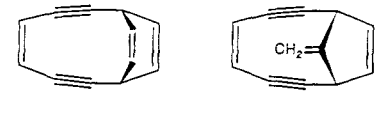
measure does not, however, apply consistently to more complex ring fusion patterns.

Consider structures **9** and **10**, an oxy analogue of calicheamicin and the corresponding bridgehead-cyclized internal Michael adduct, respectively. The cd distances differ by 0.2 A, yet **10** is predicted to cyclize with an advantage of 21.3 kcal/mol in agreement with Bredt's rule and the isolation of a number of bicyclo[7.3.1]enediynes bearing a bridgehead olefin.[1,2,7] Likewise the carbonyl isomers **11a** and **b** display a very similar diyne separation (r_{cd} = 3.41 and 3.34 A, respectively).[16] Nonetheless a substituted derivative of the former is stable at 20 °C, while the latter cannot even be detected upon generation at the same temperature.[6a,e] Equally labile is the alcohol reduction product of **11a**, namely **11c** (r_{cd} = 3.32 A). In accord, the calculated ΔE^*'s for **11b** and **11c** are diminished by 2.5 and 5.5 kcal/mol, respectively, relative to **11a** (ΔE^* = 26.1 kcal/mol) in the current model.[17] The result can be traced primarily to strain effects as surmised by Magnus and co-workers.[6a] On this basis we predict that methylene analogues **11d** and **e** will be isolable substances.[18] The approach handles the observed kinetic difference between bicyclo[7.3.1] and -[7.2.2] systems[6c] as well: ΔE^* (**11f–11g**) = 8.0 kcal/mol. Finally, unsaturated bridged analogues of the labile **8** (n = 2), for example, **12** and **13**, are predicted to show equal and considerably greater thermal stability to 1,4-diyl cyclization than **11a**. This obtains in spite of the calculated r_{cd}'s of 3.29 and 3.15, respectively.[16]



11

	X	Y	Z
a	C=O	CH_2	CH_2
b	CH_2	C=O	CH_2
c	CHOH	CH_2	CH_2
d	$C=CH_2$	CH_2	CH_2
e	$C=CH_2$	C=O	CH_2
f	CH_2		$CH_2C=O$
g	CH_2CH_2		C=O



12 **13**

In summary, the biradicaloid transition-state model affirms that strained monocyclic enediynes with short cd distances experience diminished kinetic barriers to least-motion Bergman closure by raising the energy of the ground states. Fusion of additional rings, however, introduces competing strain components not related to r_{cd}. The present treatment accommodates these effects at least

(8) Dixon, D. A.; Foster, R.; Halgren, T. A.; Lipscomb, W. N. *J. Am. Chem. Soc.* **1978**, *100*, 1359–1365. Halgren, T. A.; Kleier, D. A.; Hall, J. H. Jr.; Brown, L. D.; Lipscomb, W. N. *J. Am. Chem. Soc.* **1978**, *100*, 6595–6608.

(9) (a) Halgren, T. A. *Instruction Manual - PRDDO*; 1983. (b) Fujimoto, H. *Acc. Chem. Res.* **1987**, *20*, 448–453.

(10) Halgren, T. A.; Lipscomb, W. N. *Chem. Phys. Lett.* **1977**, *49*, 225–232.

(11) A very similar geometry for the parent transition state of **5** (3-hexene-1,5-diyne) has been obtained at the UHF/3-21G level by S. M. Ernst and K. N. Houk, private communication.

(12) Snyder, J. P.; Crans, D. *J. Am. Chem. Soc.* **1980**, *102*, 7152–7154.

(13) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127–8134. The parameters were used within MacroModel: Still, W. C.; Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Lipton, M.; Liskamp, R.; Chang, G.; Hendrickson, T.; DeGunst, F.; Hasel, W. *MacroModel V2.5*; Department of Chemistry, Columbia University: New York, NY, 10027.

(14) Cf. ref 6a and Allinger, N. L.; Pathiaseril, A. *J. Comput. Chem.* **1987**, *8*, 1225–1231.

(15) Nicolaou, K. C.; Zuccarello, G.; Ogawa, Y.; Schweiger, E. J.; Kumazawa, T. *J. Am. Chem. Soc.* **1988**, *110*, 4866–4868.

(16) To compare r_{cd} directly with previously calculated values, 0.15–0.2 A must be subtracted from the distances reported here. The difference is due to use of the softer C–C–C bending force constant[14] in the present work.

(17) For structures **11a–e** the ground-state enediyne boat six-ring is most stable as a chair in the transition state **5**.

(18) Following drafting of the manuscript, Dr. P. Magnus informed us that stable derivatives of **11d** have been prepared in his laboratory.

semiquantitatively and, where tested, permits prediction of relative ring closure rates.

Acknowledgment. Thanks go to Dr. Kosta Steliou (University of Montreal) for catalyzing and encouraging our interest in this problem. I am also grateful to Drs. S. Danishefsky (Yale), P. Magnus (University of Texas at Austin), J. Clardy (Cornell), and K. C. Nicolaou (University of Pennsylvania) for informative discussions. Dr. K. N. Houk (UCLA) kindly furnished a prepublication draft of his ab initio work on the parent **5**. Greg Tipsword (G. D. Searle) provided invaluable programming assistance.

Mechanism-Based Inhibition of Thymine Hydroxylase

Lora D. Thornburg

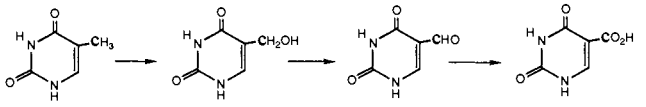
Department of Biochemistry, College of Agricultural Sciences, University of Wisconsin—Madison Madison, Wisconsin 53706

Joanne Stubbe*

Department of Chemistry Massachusetts Institute of Technology Cambridge, Massachusetts 02139

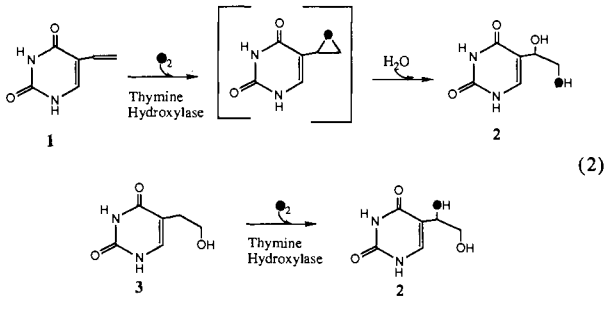
Received June 8, 1989

Thymine 7-hydroxylase (E.C. 1.14.11.6), an Fe(II)-dependent α-ketoglutarate dioxygenase, catalyzes the successive conversion of thymine to 5-hydroxymethyluracil, 5-formyluracil, and uracil-5-carboxylic acid at a single active site (eq 1). Each reaction



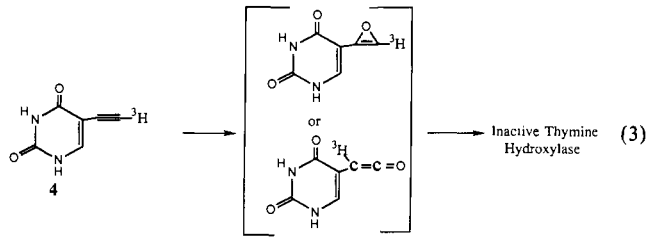
Reactions Catalyzed by Thymine Hydroxylase (1)

consumes 1 mol of O_2 and α-ketoglutarate and produces 1 mol of CO_2 and succinate.[1] While the mechanism by which non-heme Fe(II) proteins catalyze hydroxylation reactions remains to be elucidated, a reasonable proposal based on analogy with the extensively studied Fe(III) heme-dependent cytochrome P_{450}s[2,3a,b] involves an [$Fe^{II}O \leftrightarrow Fe^{IV}{=}O$] species and hydrogen atom abstraction. This postulated mechanism predicts that thymine 7-hydroxylase could catalyze the epoxidation of 5-vinyluracil (**1**, eq 2). Catalysis of an analogous reaction with 5-ethynyluracil



(2)

(**4**, eq 3) would result in oxirene or ketene production,[3b] which could lead to irreversible inhibition and covalent modification of the enzyme. This communication reports the results of studies with these compounds and demonstrates the first example of a mechanism-based inhibitor of an α-ketoglutarate dioxygenase.



(3)

Thymine 7-hydroxylase was purified from *Rhodotorula glutinis* by extensive modifications to the procedure of Abbott and coworkers.[1b,4] 5-Vinyluracil[5] was incubated with thymine 7-hydroxylase and [1-^{14}C]α-ketoglutarate under standard conditions.[6] $^{14}CO_2$ was produced at 9 μmol min^{-1} mg^{-1}, a rate of 50% that observed with thymine under the same conditions. CO_2 formation was accompanied by stoichiometric production of a new product, which could be monitored by HPLC. This product was shown to be 5-(1,2-dihydroxyethyl)uracil (**2**) by GC-MS and NMR spectroscopy and by identity with the product produced by the action of thymine 7-hydroxylase on 5-(2-hydroxyethyl)uracil (**3**).[7] Compound **2** presumably arises by enzyme-catalyzed epoxidation of 5-vinyluracil, followed by ring opening (assisted by the N-1 position of the uracil ring) and Michael addition by solvent. Attempts to isolate the proposed epoxide have thus far been unsuccessful. ^{18}O-labeling studies, however, provide strong support for this pathway. Incubation of $^{18}O_2$, α-ketoglutarate, and 5-vinyluracil with thymine 7-hydroxylase, followed by derivatization and GC-MS analysis, revealed that **2** contained a single ^{18}O atom located in the terminal OH group.[8]

Encouraged by these observations, 5-[^{3}H-ethynyl]uracil was prepared,[9] and its interaction with thymine 7-hydroxylase was investigated. Incubation of **4** with thymine 7-hydroxylase resulted in rapid time-dependent inactivation (Figure 1).[10] Inactivation

(1) (a) Liu, C. K.; Shaffer, P. M.; Slaughter, R. S. *Biochemistry* **1972**, *11*, 2172. (b) Warn-Carmer, B. J.; Macranader, L. A.; Abbott, M. T. *J. Biol. Chem.* **1983**, *258*, 10551.

(2) (a) Siegel, B. *Biorg. Chem.* **1979**, *8*, 219. (b) Hanauske-Abel, H. M.; Gunzler, V. *J. Theor. Biol.* **1982**, *94*, 421.

(3) (a) Sligar, S. G.; Murray, R. I. In *Cytochrome P-450: Structure, Mechanism and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum Press: New York, 1986; p 429. (b) Ortiz de Montellano, P. R.; Komives, E. A. *J. Biol. Chem.* **1985**, *260*, 3330.

(4) The specific activity of thymine 7-hydroxylase which is ~90% homogeneous (based on SDS gel electrophoresis) is 18 μmol min^{-1} mg^{-1}.

(5) (a) Evans, C. H.; Jones, A. S.; Walker, R. T. *Tetrahedron* **1973**, *29*, 1611. (b) Jones, A. S.; Stephenson, G. P.; Walker, R. T. *Nucleic Acids Res.* **1974**, *1*, 105.

(6) A typical assay contained in a final volume of 220 μL: 0.9 mM 5-vinyluracil, 50 mM HEPES (pH 7.5), 11 μM $FeSO_4$, 2.3 mM ascorbate, 50 μM α-ketoglutarate ([1-^{14}C]α-ketoglutarate, specific activity = 100 cpm/nmol), and 0.0035 units of thymine 7-hydroxylase. At various times, aliquots were analyzed by HPLC for products or for $^{14}CO_2$ release by standard procedures (Holme, E.; Lindstedt, S. *Biochim. Biophys. Acta* **1982**, *704*, 278).

(7) Spectral data for **2** produced from **1**: ^{1}H NMR (DMSO-d_6, 270 MHz) δ 3.24 (dd, 1 H, J = 11, 7 Hz), 3.52 (dd, 1 H, J = 11, 4 Hz), 4.40 (dd, 1 H, J = 7, 4 Hz), 7.20 (d, 1 H, J = 6 Hz), 10.74 (br d, 1 H, J = 6 Hz), 11.06 (br s, 1 H); MS of tetra-TMS derivative 445 ($M^+ - CH_3$), 357 ($M^+ - CH_3 - CH_2OTMS$). Spectral data for **2** produced from **3**: ^{1}H NMR (DMSO-d_6, 270 MHz) δ 3.29 (dd, 1 H, J = 11, 6 Hz), 3.51 (dd, 1 H, J = 11, 4 Hz), 4.41 (dd, 1 H, J = 6, 4 Hz), 7.25 (s, 1 H), 10.3–11.5 (br s).

(8) 5-Vinyluracil was converted to product under an atmosphere of either $^{18}O_2$ or $^{16}O_2$. Product was isolated by HPLC and then derivatized with 1:1 *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide/CH_3CN. Mass spectra were obtained on a Hewlett Packard Model 5987 gas chromatograph-mass spectrometer operated in the electron ionization mode with a 15 m DB-1 column. The mass spectrum of ^{16}O-labeled material contained m/z = 445 and 357. The former corresponds to $M^+ - CH_3$, and the latter to loss of the terminal CH_2–OTMS group. With ^{18}O-labeled compound, m/z = 447 and 357 were observed, indicating that ^{18}O was incorporated only into the terminal OH of the diol. To confirm the above fragmentation patterns, ^{18}O-labeled **2**, produced enzymatically from **3** and $^{18}O_2$, was isolated and derivatized. The mass spectrum contained m/z = 447 and 359, as expected.

(9) (a) Barr, P. J.; Jones, A. S.; Walker, R. T. *Nucleic Acids Res.* **1976**, *3*, 2845. (b) Kundu, N. G.; Schmitz, S. A. *J. Heterocycl. Chem.* **1982**, *19*, 463. (c) Barr, P. J.; Bohacek, L.; Jones, A. S.; Walker, R. T. *J. Labeled Compds and Radiopharm.* **1979**, *16*, 909.

0002-7863/89/1511-7632$01.50/0