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

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Mechanism-Based Inhibition of Thymine Hydroxylase

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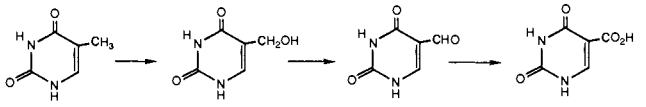
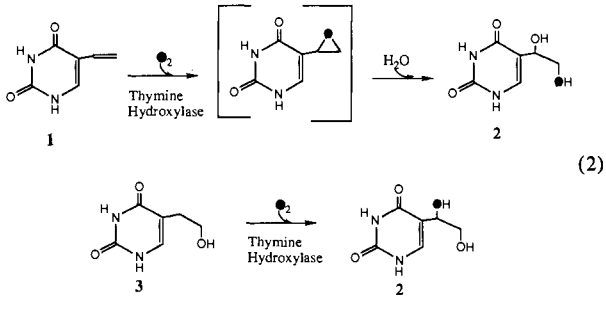
Thymine 7-hydroxylase (E.C. 1.14.11.6), an Fe(II)-dependent α-ketoglutarate dioxygenase, catalyzes the successive conversion of thymine to 5-hydroxymethyluracil, 5-formyluracil, and uracil-5-carboxylic acid at a single active site (eq 1). Each reaction



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)

(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.

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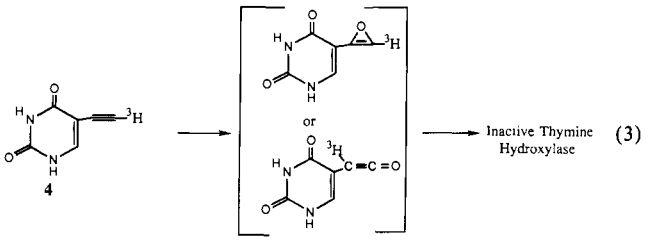
(**4**, eq 3) would result in oxirene or ketene production,[3b] which could lead to irreversible inhibition and covalent modification of the enzyme. This communication reports the results of studies with these compounds and demonstrates the first example of a mechanism-based inhibitor of an α-ketoglutarate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



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

(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.

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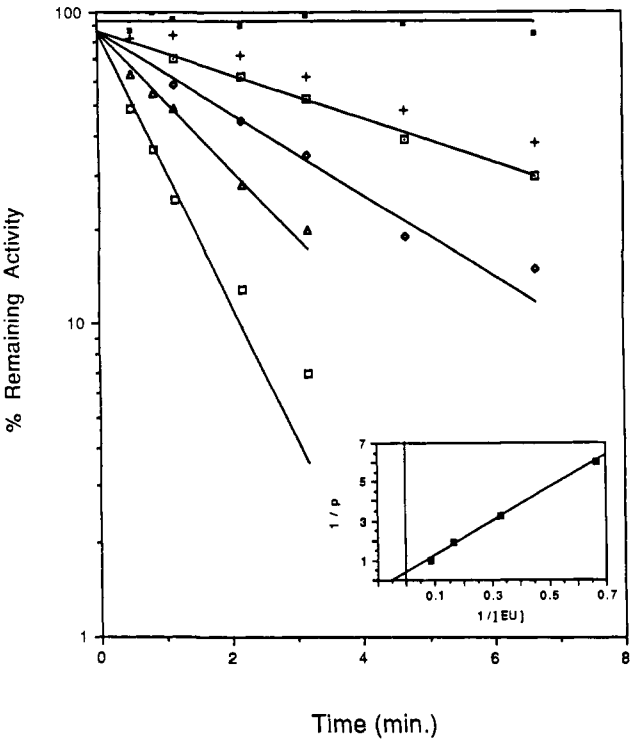

Figure 1. Time-dependent inactivation of thymine 7-hydroxylase by **4** at the following concentrations: 12 μM (□), 6 μM (△), 3 μM (◇), 1.5 μM (⊡), 0 μM (■), 12 μM without αKG (+). A double reciprocal plot of rate constant vs concentration (inset) yields a straight line (correlation coefficient = 0.999), from which K_i = 22 ± 7 μM and $t_{1/2}$ = 16 ± 5 s can be derived. Note that slow inactivation occurs in the absence of αKG and is presently under investigation.

was dependent on the presence of O_2 and α-ketoglutarate; furthermore, high concentrations of thymine provided complete protection (data not shown). To determine if the inactivation resulted from covalent modification of the protein, enzyme was incubated with ^{3}H-labeled **4** for 5 min and then passed through a Sephadex G-25 column. The amount of label bound to protein was determined by scintillation counting. Assuming a M_r of 42 kD and 90% homogeneous protein, a stoichiometry of 1 equiv of inhibitor/equiv of enzyme is observed. The labeled protein sample was diluted 10-fold into 1% SDS in 0.1% aqueous trifluoroacetic acid (pH 2.4) and dialyzed for 24 h. Ninety percent of the label remained in the dialysis bag, indicating covalent modification of the protein. These results demonstrate that 5-ethynyluracil possesses all of the criteria required of a potent mechanism-based inhibitor of thymine 7-hydroxylase. Studies are presently underway to investigate the detailed mechanism of this inactivation as well as the normal catalytic mechanism of α-ketoglutarate dioxygenases in general.

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(10) Inactivation reactions were carried out in 410 μL containing 40 mM HEPES (pH 7.5), 0.54 mM α-ketoglutarate, 12 μM $FeSO_4$, 2.4 mM ascorbate, 22 μg (0.006 units) thymine 7-hydroxylase, 1 mg mL^{-1} BSA, 2% glycerol, 0.2% DMSO, and variable amounts of 5-ethynyluracil (0–12 μM). At various times, a 10-μL aliquot was diluted into a standard assay mixture (200 μL) containing 1 mg/mL BSA, [2-^{14}C]thymine (1100 cpm/nmol), 40 mM HEPES (pH 7.5), 0.54 mM α-ketoglutarate, 12 μM $FeSO_4$, and 2.4 mM ascorbate. After 5 min, the assays were quenched with 40 μL of 10% perchloric acid; chloroform (50 μL) was added, the samples were neutralized with 10 μL 6 N KOH in 1 M Tris, and the products were analyzed by HPLC.

Synthesis and Characterization of a Novel Bimetallic μ-Malonyl Complex. The First X-ray Crystal Structure of Alkali Metal Chelation by a Neutral Malonyl Compound

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Carbon-bound malonyl (β-oxoacyl) transition-metal complexes ($MCOCH_2COR$), in contrast to simple metal acyls ($MCOCH_3$), are remarkably elusive compounds.[1] The properties and reactivity of this ligand class are expected to vary considerably from those of typical metal acyls due to the increased acidity of the α-hydrogens, the increased lability of the carbon–carbon bonds, and the potential of the malonyl oxygens to chelate to an additional metal. We recently developed a synthetic route into bimetallic μ-malonyl complexes, which are stabilized by chelation of a malonyl oxygen to one of the transition metals. Thus reaction of $(\eta^5\text{-}C_5Me_5)Re(NO)(PPh_3)(COCH_2Li)$, **1**,[2] and $Re(CO)_5(O\text{-}SO_2CF_3)$, **2**, led to isolation of $(\eta^5\text{-}C_5Me_5)Re(NO)(PPh_3)(\mu\text{-}COCH_2CO)_4Re(CO)_4$, **3**, in 71% yield.[3] We wish to report here the synthesis and characterization of a novel μ-malonyl complex, $(\eta^5\text{-}C_5Me_5)Re(NO)(PPh_3)(\mu\text{-}COCH_2CO)Re(CO)_4(PMe_3)\cdot Li^+\text{-}OSO_2CF_3^-$, **4**, in which the neutral 1,3-dicarbonyl bridge serves as a bidentate chelate to an alkali metal cation. The X-ray crystallographic characterization of **4** represents the first solid-state structure of an alkali metal ion chelated by both carbonyl oxygens of a neutral malonyl compound.

When the reaction of rhenaenolate **1** and triflate **2** is followed by low-temperature $^{31}P\{^1H\}$ NMR spectroscopy, evidence is obtained for clean, quantitative formation of an intermediate species which, upon warming to 23 °C, undergoes conversion to **3**. Addition of excess PMe_3 to solutions of the intermediate at −78 °C generates the lithium-chelated complex $(\eta^5\text{-}C_5Me_5)Re(NO)(PPh_3)(COCH_2CO)Re(CO)_4(PMe_3)\cdot Li^+OSO_2CF_3^-$, **4**, in 45% isolated yield. The malonyl ligand in **4** exhibits an extremely large ^{1}H NMR chemical shift difference ($CDCl_3$) for the diastereotopic methylene hydrogens [δ 1.48 (d, J = 14.6 Hz, 1 H), 5.18 (d, J = 14.7 Hz, 1 H)]. In the ^{1}H NMR spectrum of the isotopically enriched complex $(\eta^5\text{-}C_5Me_5)Re(NO)(PPh_3)(^{13}COCH_2CO)Re(CO)_4(PMe_3)\cdot Li^+OSO_2CF_3^-$, **4**-^{13}**CO**, the methylene resonance at δ 1.48 is observed as a doublet of doublets ($^2J_{HH}$ = 14.5 Hz, $^2J_{CH}$ = 5.5 Hz), whereas the 5.18 resonance remains a doublet ($^2J_{HH}$ = 14.7 Hz). For comparison we prepared the corresponding ^{13}C-enriched complex $(\eta^5\text{-}C_5Me_5)Re(NO)(PPh_3)(^{13}COCH_2CO)Re(CO)_4$, **3**-^{13}**CO**, which exhibits resonances in the ^{1}H NMR spectrum ($CDCl_3$) at δ 3.10 ($^2J_{HH}$ = 20.6 Hz, $^2J_{CH}$ = 1.6 Hz) and 2.49 (dd, $^2J_{CH}$ = 2.5 Hz). In the solid-state structure of **3**, the malonyl ligand is incorporated into a nearly planar five-membered ring. We believe that the differential $^2J_{CH}$ coupling and the large chemical shift difference in the ^{1}H NMR

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