Chemical Racemization of 5-Benzylhydantoin

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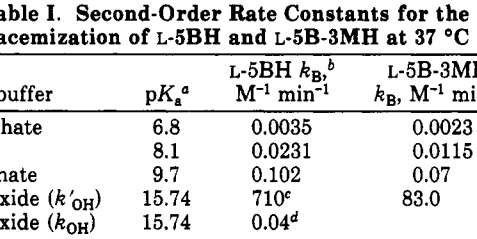
The chemical racemization of L-5-benzylhydantoin (L-5BH) at 37 °C has been investigated in H_2O from pH 6 to 14. The pH–rate profile demonstrates hydroxide catalysis on both the free base and free acid form (or the kinetically equivalent water catalysis on the free base form) of L-5BH. In addition the reaction is subject to buffer catalysis by phosphate, Tris, and carbonate. Evidence is presented for a mechanism involving general base catalysis based upon comparison of the second-order buffer-catalyzed rate constants for L-5BH with those obtained for L-5-benzyl-3-methylhydantoin (L-5B-3MH). A Brønsted plot yields a $\beta_{gb} = 0.59$ for L-5BH and $\beta_{gb} = 0.51$ for L-5B-3MH, implying that the proton at carbon 5 is approximately halfway between the hydantoin and the buffer species in the transition state of the racemization reaction.

The production of optically active amino acids from inexpensive racemic starting materials is of great interest.[1] In particular, the synthesis of L-phenylalanine has attracted much attention recently due to its use in the peptide sweetener aspartame.[2] One attractive approach to L-phenylalanine[3] involves the stereospecific enzymatic hydrolysis of D,L-5-benzylhydantoin (D,L-5BH), an inexpensive precursor that can be prepared from phenylacetaldehyde by the Bucherer–Bergs synthesis.[4] The enzymatic hydrolysis of racemic hydantoins proceeds via either the *N*-carbamoyl D- or L-amino acid. This can be followed by enzymatic or chemical hydrolysis to the subsequent D- or L-amino acid.[3,5] While enzymatic D,L-5BH racemase activity has been observed,[3a,b] the chemical racemization of D-5BH coupled with a specific L-5BH hydantoinase and *N*-carbamoyl-L-phenylalanine hydrolase represents the basis for a potential commercial process. The chemistry of hydantoins has been well studied.[6] In particular, base-catalyzed racemization has been known for many years and has been proposed to occur via enolate tautomers.[7] However, the specific mechanism of racemization by lyate species has not been addressed. The purpose of this study is to present evidence concerning the lyate and buffer-catalyzed racemization of 5-BH (Scheme I).

Results and Discussion

The chemical racemization of L(or D-)-5BH was investigated over the pH range 6–14 in H_2O at 37 °C. No detectable hydrolysis of 5-BH was observed under these conditions. The pseudo-first-order rate constant of racemization k_{obs} is the sum of the forward and reverse rate constants ($k_1 + k_{-1}$) (Scheme I) or twice the interconversion rate of either optical form to its enantiomer.[8] In addition to lyate species catalysis, there is also substantial catalysis

Scheme I



L-5BH D-5BH

Table I. Second-Order Rate Constants for the Racemization of L-5BH and L-5B-3MH at 37 °C

buffer	pK_a[a]	L-5BH k_B,[b] M^{-1} min^{-1}	L-5B-3MH k_B, M^{-1} min^{-1}
phosphate	6.8	0.0035	0.0023
Tris	8.1	0.0231	0.0115
carbonate	9.7	0.102	0.07
hydroxide (k'_{OH})	15.74	710[c]	83.0
hydroxide (k_{OH})	15.74	0.04[d]	

[a] Determined by pH at half-neutralization. [b] Calculated for general base catalysis on the free-acid form of L-5BH. [c] Calculated from $k'_{OH} = K_a k_{H_2O}/K_w$ where k_{H_2O} is determined from Figure 1 between pH 9 and 12. [d] Represents hydroxide catalysis on the free-base form of L-5BH.

observed by various buffer species. The dependence of k_{obs} on pH at zero buffer concentration is shown in Figure 1. Values were obtained from the ordinate intercept of plots of k_{obs} versus total buffer concentration.

The pH–rate profile for 5-BH racemization shows a hydroxide catalyzed term at high pH as well as a term dependent on the ionization state of the hydantoin with a kinetically determined pK_a of ca. 8.7. Spectrophotometric titration of 5-BH yielded a pK_a of 8.65 ± 0.10, consistent with values published for other hydantoins[9] and the kinetically determined pK_a above. The data from the racemization of 5-BH is consistent with a rate law described by the kinetically equivalent equations 1 or 2

$$k_{obs} = (k'_{OH}a_{OH} + k_B[\text{Buffer}]_B)\alpha + k_{OH}a_{OH}\beta \quad (1)$$

$$k_{obs} = (k_{H_2O} + k_{OH}a_{OH} + k_{BH}[\text{Buffer}]_{BH})\beta \quad (2)$$

where k'_{OH} and k_B represent rate constants for hydroxide (a_{OH}) and the free base form of the buffer ($[\text{Buffer}]_B$) acting on the neutral free acid form of 5-BH, α ($= a_H/(K_a + a_H)$); k_{H_2O}, k_{OH}, and k_{BH} represent the rate constants associated with water, hydroxide, and the general acid form of the buffer ($[\text{Buffer}]_{BH}$) acting on the anionic free base form of 5-BH, β ($= K_a/(K_a + a_H)$); K_a is the dissociation constant for 5-BH.

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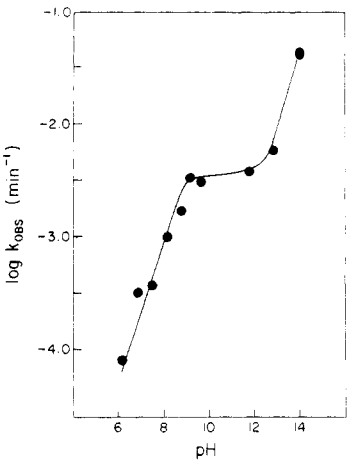

Figure 1. Dependence on pH of the observed pseudo-first-order rate constant for racemization of L-5BH extrapolated to zero buffer concentration at 37 °C. The line is calculated from eq 1 using the data in Table I and $pK_a = 8.65$.

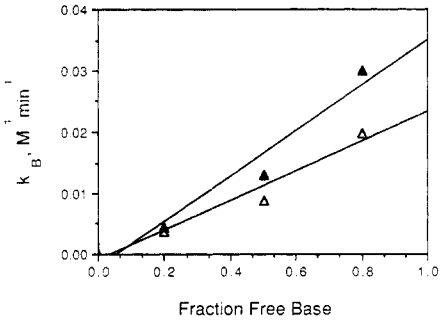

Figure 2. Dependence of the apparent second-order rate constant, k_B [$=(k_{obs} - k'_{OH}a_{OH}\alpha + k_{OH}a_{OH}\beta))/([Buffer]\alpha)$] for the buffer-catalyzed racemization of the free-acid form of L-5BH on the mole fraction of buffer in the free base form. [△, k_B (Tris); ▲, $10k_B$ (phosphate)].

The curve in Figure 1 is calculated from eq 1 using the values for the second-order rate constants, which are listed in Table I. The buffer-catalyzed term in eq 1 is obtained from plots of k_{obs} versus buffer concentration followed by replots of the slopes, divided by the mole fraction free acid form of 5-BH (α), versus the mole fraction of buffer in the free base form (Figure 2). Values for the second-order rate constants for general base buffer catalysis, k_B, are obtained from the ordinate intercept where the fraction free base = 1. Alternatively, the curve in Figure 1 can be calculated from the kinetically indistinguishable eq 2; the term k_{BH} is simple k_BK_b/K_a, where K_b is the dissociation constant of the buffer species.

In order to distinguish between the kinetically equivalent general base catalysis on the acidic neutral form of 5-BH (eq 1) or general acid catalysis on the anionic free base form of 5-BH (eq 2), a study was undertaken in which the ionizable imide proton was replaced by a methyl group. The racemization of L-5-benzyl-3-methylhydantoin (L-5B-3MH) is also subject to buffer catalysis. Replots of the data clearly shown that L-5B-3MH is subject to general base catalysis. The rate law is described by eq 3

$$k_{obs} = k'_{OH}a_{OH} + k_B[Buffer]_B \quad (3)$$

Values for the second-order rate constant for buffer catalysis on L-5B-3MH are also listed in Table I. It is interesting to note that the magnitude of the second-order rate constants for the different buffer species, when calculated as general base catalysts, is essentially the same for L-5B-3MH and the free acid form of L-5BH. In addition, a Brønsted plot of the data reveals a $\beta_{gb} = 0.59$ for L-5BH and $\beta_{gb} = 0.51$ for L-5B-3MH (Figure 3). This data is consistent with a mechanism of general base catalysis on the uncharged form of L-5BH, described by eq 1, where the position of the proton at C5 in the transition state is approximately halfway between the buffer species and chiral carbon on the hydantoin.

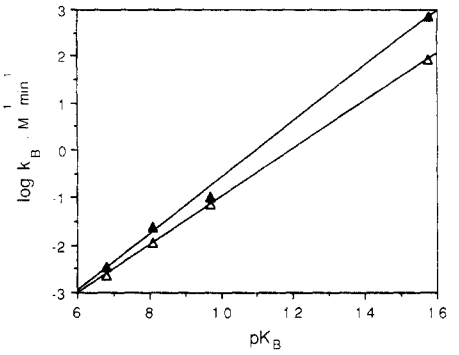

Figure 3. Brønsted plot showing the dependence of the second-order rate constant for racemization of L-5BH (▲) and L-5B-3MH (△) on the pK_a of the buffer species. A least-squares linear regression analysis yields slopes of 0.59 and 0.51, respectively.

A mechanism involving general base buffer catalysis has also been proposed to explain the racemization of 5-phenylhydantoin,[10] a biosynthetic intermediate that has been observed in the N-deethylation of ethotoin in mammalian species.[9a] In this case the observed rate constants for k_B (phosphate) and k'_{OH} are approximately 170- and 16-fold larger, respectively, than those observed for L-5BH. This is presumably due to the increased transition-state stabilization of the developing carbanion at C5 by the phenyl group as compared to the benzyl group. This explanation is also consistent with the relatively slow racemization rates observed for 5-methylhydantoin.[9b]

The effect of the charge state of the hydantoin ring (i.e. ionization of the imide proton) on the racemization rate can be calculated by comparing the two values of k'_{OH} for the ionized and nonionized species. A rate enhancement of 1.8×10^4, which corresponds to a free energy of activation of 6.1 kcal/mol, is observed for the neutral nonionized hydantoin. Deprotonation of the imide proton, therefore, leads to a resonance delocalized species, which, as expected, is considerably more resistant to racemization.

In summary, the chemical racemization of either enantiomer of 5-BH demonstrates general base catalysis on the neutral form as well as hydroxide catalysis on the ionized form of the hydantoin. The absolute rates for this reaction could support a recycle process involving stereospecific enzymatic hydrolysis of D,L-5BH to L-phenylalanine followed by separation and chemical racemization of the remaining D-5BH at either high pH or high buffer concentrations.

Experimental Section

Chemicals and buffers were of the highest commercial grade and used without further purification. Water was doubly distilled and deionized. Melting points were determined on a Büchi 510 apparatus and are uncorrected. Mass spectra (electron impact) were obtained on a Hewlett-Packard 5985B spectrophotometer. Optical rotations were measured using a Rudolf Autopol II polarimeter. High-performance liquid chromatography (HPLC) was performed using a Waters 510 pump, a Waters 681 UV detector at 215 nm, a Hewlett-Packard 3392A integrator, an Alltech 25 cm × 4.6 mm 10 μ C8 column, and a mobile phase of MeOH–

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0.02% HOAc (90:10) at 1 mL/min. Retention times for D,L-5BH, *N*-carbamoyl-D,L-phenylalanine, and D,L-phenylalanine are 15.8, 13.9, and 5.3 min, respectively. All UV measurements were taken on a Hewlett-Packard 8451 diode array spectrophotometer. pH measurements were made at 25 °C using a Corning 125 pH meter with a Corning 476223 combination electrode.

Synthesis. Optically pure L- or D-5-benzylhydantoin were synthesized in 70% yields from the corresponding amino acid and potassium cyanate according to the method of Finkbeiner.[11] L-5BH: mp 173 °C (lit.[12] mp 181–183 °C); MS *m/e* 190 (M^+), 160, 146, 128, 117, 103, 91; $[\alpha]^{24}_D$ = −93.7° (*c* = 1, acetone), ϵ_{240} = 0.45 mM^{-1} (H_2O). Identical parameters were observed for D-5BH except that $[\alpha]^{24}_D$ = +94.0° (*c* = 1, acetone).

Optically pure *N*-carbamoyl-L- and -D-phenylalanine were prepared in 60% yields from the corresponding amino acid as previously described.[13] *N*-Carbamoyl-L-phenylalanine: mp 181 °C (recrystallized from MeOH–H_2O) (lit.[13] mp 192–193 °C); $[\alpha]^{24}_D$ = +40° (*c* = 0.2, MeOH), ϵ_{240} = 0.085 mM^{-1} (H_2O). *N*-Carbamoyl-D-phenylalanine: mp 179 °C (recrystallized from MeOH–H_2O); $[\alpha]^{24}_D$ = −40° (*c* = 0.2, MeOH).

L-5-Benzyl-3-methylhydantoin was synthesized from L-phenylalanine and methylisocyanate according to the method of Dudley and Bius[14] in an overall yield of 50%. The intermediate L-2-benzyl-5-methylhydantoin acid that precipitated at pH 2 was isolated and cyclized to optically active L-5-benzyl-3-methylhydantoin by refluxing in 0.5 N HCl for 30 min. The white crystals were washed with cold water and dried in vacuo: mp 166–167 °C; MS *m/e* 204 (M^+), 160, 146, 117, 91; $[\alpha]^{24}_D$ = −113° (*c* = 1.0, acetone).

Kinetics. The racemization of L(or D-)-BH_2 was measured by following the change in circular dichroism, θ, at 230 nm with respect to time as measured on a JASCO J-500A spectropolarimeter. The cuvette was housed in a thermostable cell maintained at 37 °C by a Lauda circulatory water bath. Stock solutions of optically active L- or D-5BH in H_2O at 4 °C were added to preequilibrated buffer to give 0.1 mg/mL solutions. Aliquots were taken from stoppered glass vials at 37 °C for slower reactions. Reactions were followed for at least 3 half-lives; no optical activity is observed after complete racemization. The observed pseudo-first-order rate constants for racemization k_{obs} were calculated from the slopes of linear plots of ln $(\theta_t - \theta_\infty)$ against time. Plots were generally linear to 3 half-lives. Buffers employed (0.25–1 M) were phosphate, Tris, carbonate, and hydroxide. General acid–base catalysis was observed for these species. The pH–rate profile for lyate species was determined by extrapolation of the racemization rate to zero buffer concentration.

pK_a Determination. The pK_a for 5-BH (8.65 ± 0.10) was determined by spectrophotometric titration at 230 mm where ϵ_{230} = 1.45 mM^{-1} for the neutral free acid form and ϵ_{230} = 3.04 mM^{-1} for the anionic free base form.

Product Determination. There was no detectable hydrolysis of 5-BH during the course of the racemization studies as evidenced by (a) no observable change in UV absorbance at 240 nm and (b) only one peak corresponding to 5-BH detected by HPLC.

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Notes

Antimony(III) Chloride Exerts Potent Catalysis of the Conversion of Sulfoxides to α-Fluoro Thioethers with (Diethylamino)sulfur Trifluoride

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Intensive efforts on the synthesis and biological evaluation of various types of organofluorine compounds have been reviewed.[1-3] The size and electronegativity of the fluorine atom make isosteres of important biological molecules in which hydrogen has been replaced by fluorine of significant interest. We now report details of the dramatic catalysis by antimony(III) chloride for the conversion of sulfoxides to α-fluoro thioethers with (diethylamino)-sulfur trifluoride (DAST).

Zupan[4] and Janzen and co-workers[5] reported syntheses of α-fluoro thioethers by treatment of thioethers with xenon difluoride. Rigorously dried potassium fluoride in the presence of 18-crown-6 ethers has been employed to convert α-chloro to α-fluoro thioethers.[6] McCarthy et al. discovered that DAST converted sulfoxides to α-fluoro thioethers.[7] They noted that *p*-methoxyphenyl thioethers were much more reactive than phenyl thioethers, and the reaction was catalyzed by zinc(II) iodide. Thioethers have since been α-fluorinated with *N*-fluoropyridinium triflate,[8] and dithioacetals have been converted to α-fluoro thioethers with mercury(II) fluoride.[9]

Zupan[4] and McCarthy and co-workers[7] reported syntheses of α,α-difluoro thioethers from α-fluoro thioethers and α-fluoro sulfoxides, respectively. Some α-(fluoromethyl) thioethers have been converted[7,9] to α-fluoro sulfoxides,[10] α-fluoro sulfones,[11] and α-fluoro sulfoximines,[12] precursors for "fluoromethylene-Wittig" reag-

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