Racemization and Intramolecular Nucleophilic Substitution Reactions of Ibutilide

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Received August 12, 1991, from the *Drug Delivery Research and Development Unit and §Drug Metabolism Research Unit, The Upjohn Company, Kalamazoo, MI 49001, and ‡Pfizer Central Research, Building 156, Groton, CT 06340. Accepted for publication February 19, 1992.

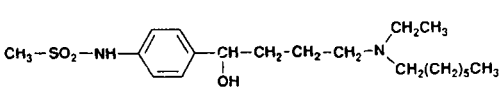
Abstract □ The kinetics and mechanisms of the racemization and cyclization reactions of ibutilide are described. The cyclization reaction yields a bell-shaped rate–pH curve consistent with a change in rate-determining step. It is hypothesized that the hydroxyl group leaves to form a carbocation intermediate; this is followed by nucleophilic attack by the amine. This mechanism is supported by kinetic analysis, aniline trapping of carbocation intermediate, and observation of all four stereoisomers of the resulting quaternary ammonium compound. Whereas racemization can also progress through the carbocation intermediate, a direct S_N2 mechanism appears to be the major route for the racemization reaction.

The racemization of pharmacologically active agents is of interest, because enantiomers often have significantly different absorption, distribution, metabolism, and excretion, in addition to differing pharmacologic action.[1] An example of a compound that rapidly racemizes in aqueous solution is *l*-epinephrine (adrenalin),[2] the *d* form of which has significantly less adrenergic activity. Epinephrine is also of interest because it undergoes nucleophilic attack by sulfite ions (commonly added as an antioxidant) at the chiral carbon.[2] The dextrorotatory enantiomer of ibutilide (1; U-70226), a racemic antiarrhythmic agent, prolongs the QT interval (the time between the Q and T waves in an electrocardiogram) to a greater extent than the levorotatory enantiomer.[3] Previous studies have shown that ibutilide degrades to form a cyclic quaternary ammonium product (2; U-87473)[4,5] and that the enantiomers of ibutilide may racemize in aqueous solution.[6] The purpose of the present report is to characterize the kinetics and mechanisms of these reactions.

Experimental Section

Chromatography—Three separate chromatographic systems were used. Sample concentration was analyzed with a 30-cm-long μBondpak C_{18} column (Waters Associates, Milford, MA), a 3-cm-long Spheri-5 RP-18 guard column (Brownlee Labs, Santa Clara, CA), a Perkin Elmer Series 410 pump (Danbury, CT), a Perkin Elmer LC-235 photodiode array detector, a Nelson Series 2600 integration software (Cupertino, CA), and a Perkin Elmer ISS-100 autosampler. The mobile phase consisted of 40–55% acetonitrile, with the remaining portion made up with 0.05 M potassium phosphate monobasic (the lower percent organic constituent was required for samples containing aniline). The flow rate was 2 mL/min, an injection volume of 20 μL was used, and UV detection was carried out at 230 nm. Ibutilide concentration was quantitated by using external standards.

The ratios of the two ibutilide enantiomers in solution were determined by a chromatographic procedure previously described by Hsu and Walters.[6] Samples were derivatized with 1-naphthylisothiocyanate, and the enantiomers were separated on a chiral Pirkle column (covalent 3,5-dinitrobenzoyl-D-phenylglycine; Regis, Morton Grove, IL). The chromatographic system consisted of a Waters 710B Wisp autosampler, an LDC Milton Roy SM4000 detector (Riviera Beach, FL), a Nelson Series 2600 integration software, and a Beckman model 110A pump (Fullerton, CA).



Ibutilide (1)

The four stereoisomers of 2 were separated by using a 4-mm-diameter Chiral-AGP 100-mm-long column (J. T. Baker, Phillipsburg, NJ). The mobile phase (flow rate of 1 mL/min) consisted of either 4% 2-propanol or 8% acetonitrile in 0.01 M pH 7.0 sodium phosphate buffer. The chromatographic system consisted of a Perkin Elmer ISS-100 autosampler, a Spectroflow 783 UV detector (ABI, Ramsey, NJ), a Beckman model 110A pump, and a Harris H100 computer (Fort Lauderdale, FL) with custom interface and software (Upjohn, Kalamazoo, MI). An injection volume of 200 μL was used, and UV detection was carried out at 230 nm.

Potentiometric Titration—The acid dissociation constants of ibutilide were determined at 25 °C with a Metrohm 655 automated dispenser and a 672 microprocessor (Metrohm AG, Switzerland) by a previously described method.[7] The concentration of ibutilide was 0.001 M, the ionic strength was adjusted to 0.1 M with sodium chloride, and the titrant was 0.1 M sodium hydroxide.

Sample Preparation and Study Conditions—Ibutilide (*N*-[4-[4-(ethylheptylamino)-1-hydroxybutyl]phenyl]methanesulfonamide) and its two enantiomers were prepared by the Chemical Research Preparations Unit of the Upjohn Company and were all >98% pure. All other chemicals used were reagent grade. The drug concentration of all samples was 5.65×10^{-4} M, and all samples were prepared in duplicate. The effect of pH was determined at 25 and 70 °C for the racemization and cyclization reactions, respectively. Both reactions were carried out in 0.15 M ionic strength buffer solutions (calculated with BUFCALC[8]). The pH of the solutions was examined periodically to ensure that no pH change >0.2 pH units had occurred. Kinetic analysis was performed on the combined data for both enantiomers. All other studies were performed in a pH 4.6, isotonic, 0.0346 M acetate buffer at 80 °C. For the trapping experiments, aniline was added at various concentrations to 5.65×10^{-4} M solutions of ibutilide and its enantiomers. Quantification of 2 was performed by applying a previously determined response factor of 1.2 (relative to ibutilide).[5] The amount of aniline conjugate was then calculated by difference.

Results and Discussion

Effect of pH on Racemization and Formation of Cyclic Quaternary Ammonium Product—Whereas the rate of racemization could be treated as a first-order process by using initial rate kinetics, it is desirable to take all stability data into account. First, consider the general equilibrium situation for two species A and B:

$$dx/dt = k(A_0 - x) - k'(B_0 + x) \quad (1)$$

In eq 1, k and k' are the forward and reverse rate constants; x is the extent of the reaction; t is time; A and B are the concentrations of A and B, respectively, and the subscript 0

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indicates the concentration at the initial time. For racemization, $k = k'$. The equation is further simplified by assuming that only one species is present at the initial time point. These assumptions result in eq 2:

$$dB/dt = k_{rac}(A_0 - 2B) \quad (2)$$

In eq 2, k_{rac} is the rate constant of racemization. Integration of eq 2 yields the following:

$$(A_0 - 2B)/A_0 = \exp(-2k_{rac}t) \quad (3)$$

(Figure 1 presents typical data.) The racemization of the two enantiomers of ibutilide as a function of pH at 25 °C is consistent with a specific-acid-catalyzed mechanism (for which the rate constant is k_H) in addition to an uncatalyzed mechanism (for which the rate constant is k_{H_2O}) (Figure 2). Nonlinear fitting[9] of these data with eq 4 gives $k_H = 0.718$ (0.268) $M^{-1} \cdot day^{-1}$ and $k_{H_2O} = 5.6\ (1.0) \times 10^{-5}\ day^{-1}$ [95% confidence interval (CI) in parentheses].

$$k_{rac} = k_H[H^+] + k_{H_2O} \quad (4)$$

A base-catalyzed mechanism involving a concerted loss of the sulfonanilide proton and the hydroxyl group does not appear to play a significant role.

The first-order rate constant for the loss of ibutilide is shown as a function of pH in Figure 3. This study was carried out at 70 °C, because the cyclization reaction proceeds slowly at 25 °C. Compound 2 accounts for all of the loss of ibutilide except at pH 2, at which pH other degradation products are also observed.[5] A maximum rate is observed at pH ~6. A bell-shaped curve is consistent with a change in the rate-determining step as a function of pH. We think that the hydroxyl group leaves to form a carbocation intermediate; this is followed by nucleophilic attack by the amine (Scheme I). With the loss of the hydroxyl group, the compound is prone to acid catalysis (as evidenced by the racemization reaction), which may account for the decreased rate of the reaction at pH > 6. The increasing rate of reaction as a function of pH (pH < 6) is likely due to the increasing fraction of the unprotonated amine [pK_a (K_a is the acid dissociation constant) = 10.5; standard error = 0.008] as the pH is raised. However, the interpretation of the cyclization kinetics is somewhat complicated at pH > 6 because of the ionization of the sulfonanilide (pK_a = 8.36; standard error = 0.01). To further study these reactions, we concentrated on pH 4.6 solutions. In addition to being in a more pharmaceutically relevant region (on the basis of solubility and stability arguments), this pH allows one to ignore the ionization of the sulfonanilide.

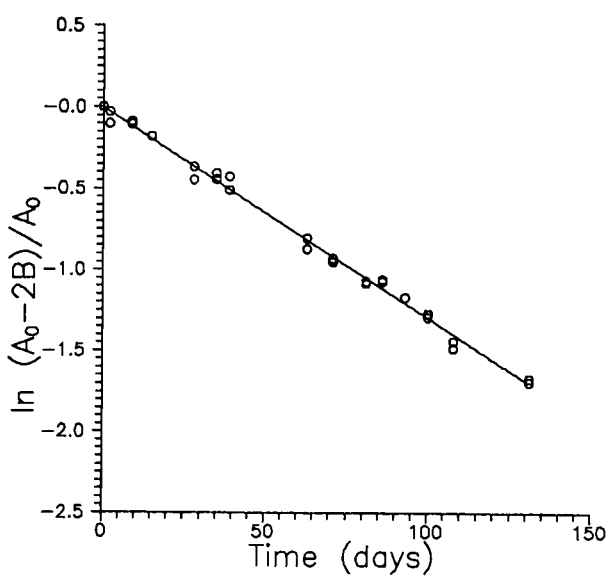


Figure 1—Data for racemization of ibutilide enantiomers fitted to eq 3 (25 °C, pH 2, ionic strength of 0.15 M).

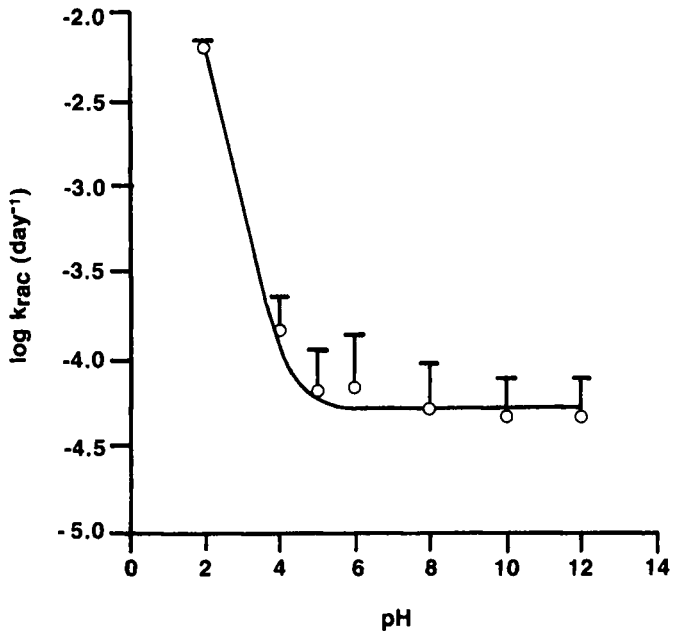


Figure 2—Rate of racemization as a function of pH at 25 °C (ionic strength of 0.15 M). The line represents the theoretical fit described by eq 4, and the error bars represent 95% CIs.

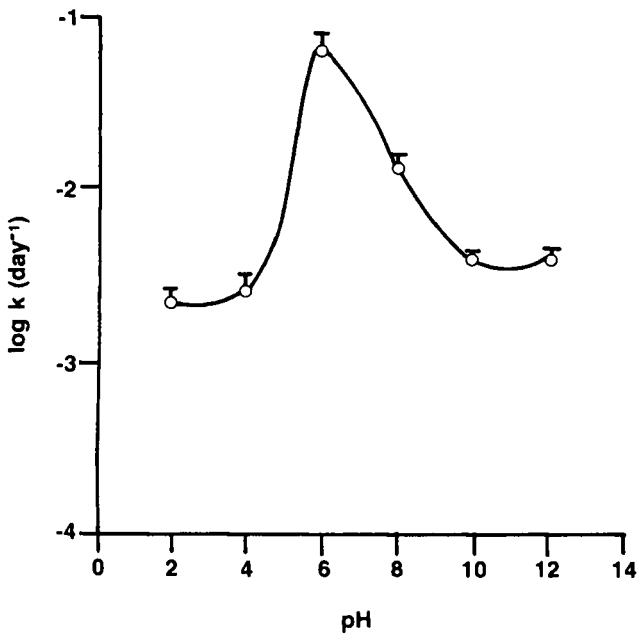
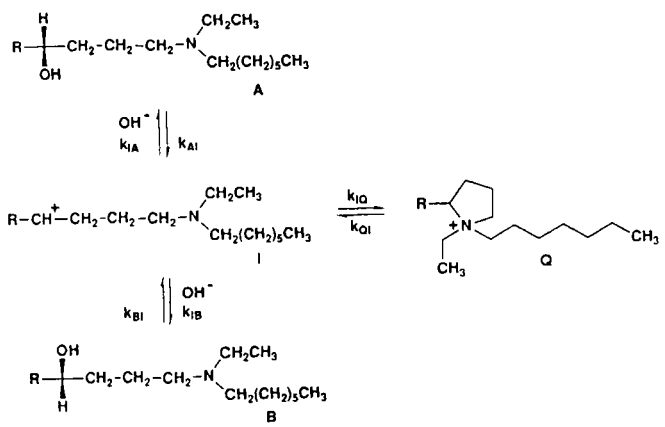


Figure 3—Rate of the formation of the cyclic quaternary ammonium product as a function of pH at 70 °C (ionic strength of 0.15 M). The error bars represent 95% CIs.

Characterization of 2 by Chiral High-Performance Liquid Chromatography (HPLC)—Achiral HPLC of heat-treated ibutilide samples yields two peaks for 2 with similar corresponding concentrations (peak area ratio, ~6:5). Each



Scheme I—Proposed mechanism for the racemization and cyclization of ibutilide. Compound **2** is designated Q in this scheme.

peak corresponds to a pair of enantiomers, as verified with the Chiral-AGP chromatographic system. This same system was used to analyze 2 that was generated from the degradation of an individual ibutilide enantiomer at 70 °C. If 2 is formed via an S_N2-type reaction, only two stereoisomers would be expected. However, if the reaction proceeds through a carbocation intermediate (Scheme I), four stereoisomers are expected. Each peak observed by achiral HPLC corresponds to two enantiomers in equal amounts (peak area ratio = 0.97; standard deviation = 0.03). Thus, the intramolecular reaction appears to proceed exclusively through an S_N1-type mechanism.

Simultaneous Racemization and Formation of 2—The mechanism in Scheme I may be described kinetically by eqs 5–8:

$$dA/dt = -k_{AI}A + k_{IA}I \quad (5)$$

$$dB/dt = -k_{BI}B + k_{IB}I \quad (6)$$

$$dQ/dt = -k_{QI}Q + k_{IQ}I \quad (7)$$

$$dI/dt = k_{AI}A + k_{BI}B + k_{QI}Q - I(k_{IA} + k_{IB} + k_{IQ}) \quad (8)$$

In eqs 5–8, A and B are the concentrations of the two enantiomers, I is the concentration of the carbocation intermediate, and Q is the concentration of the quaternary ammonium degradation product, 2. Because A and B are enantiomers, one may assume that $k_{AI} = k_{BI}$ and $k_{IA} = k_{IB}$. Nonlinear regression[9] analysis was used to analyze plots of the concentrations of A, B, and Q (which is compound 2) as a function of time at 80 °C. These data and the estimated rate constants are shown in Figure 4 and Table I, respectively. As expected, there is good agreement for the rates of the two enantiomers. The data appear to be consistent with eqs 5–8, a result suggesting that racemization proceeds through the same intermediate as the formation of the cyclic quaternary ammonium product. When these same data are analyzed by a model that ignores the chirality of the reaction ($R = A + B$), an excellent fit is also observed (Figure 5):

$$dR/dt = -k_{RI}R + k_{IR}I \quad (9)$$

$$dQ/dt = -k_{QI}Q + k_{IQ}I \quad (10)$$

$$dI/dt = k_{RI}R + k_{QI}Q - I(k_{IR} + k_{IQ}) \quad (11)$$

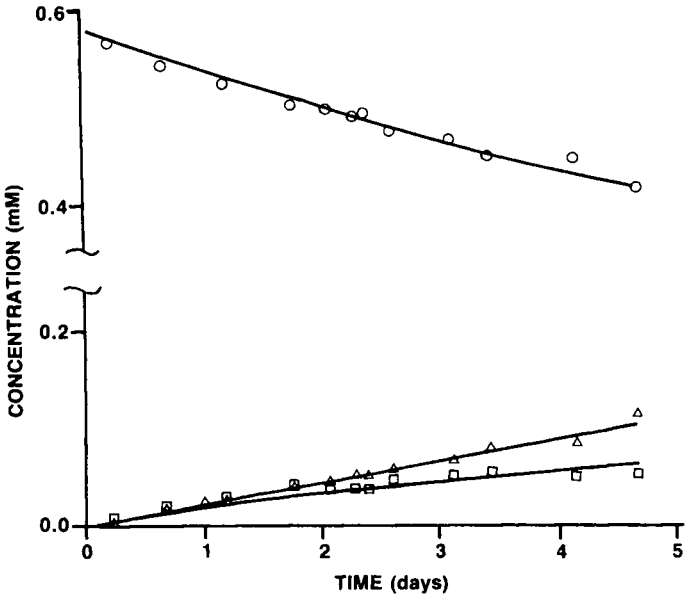


Figure 4—Concentrations of the original enantiomer A (○), the newly formed enantiomer B (□), and the cyclic quaternary ammonium product Q (△) as a function of time at pH 4.6 and 80 °C. The lines represent the fit of experimental data to eqs 5–8.

Table I—Estimated Reaction Rates[a]

Rate Constant	(+)-Enantiomer	(−)-Enantiomer
k_{AI}	0.110 (0.005)	0.106 (0.006)
k_{IA}	7.83 (11.5)	6.75 (13.5)
k_{IQ}	9.74 (14.0)	9.46 (18.2)
k_{QI}	8.4×10^{-5} (0.16)	1.7×10^{-5} (0.16)

[a] Determined at 80 °C; unit is day^{-1}; 95% univariate CIs in parentheses.

The values for the rate constants k_{RI}, k_{IR}, k_{IQ}, and k_{QI} were 0.0555 (0.0125), 6.19 (1.81), 20.5 (16.3), and 1.0×10^{-5} (0.19) day^{-1}, respectively (95% CI in parentheses). The rate constant k_{RI} is significantly lower than k_{AI}. This result suggests that racemization may be occurring via a direct S_N2 mechanism, in addition to the mechanism involving a carbocation intermediate. The data also suggest that conversion of the quaternary ammonium product to ibutilide occurs at a slow rate under the conditions of the present study.

Attempts were made to fit the data to a model that includes the rate constants in Scheme I and the rate constants for the direct S_N2 racemization reaction. Whereas this model fit the data well, the estimated rate constants were very dependent on the initial estimates. Furthermore, the confidence limits of the resulting rate constants were relatively large. Thus, kinetic analysis alone was unable to provide evidence supporting or opposing the S_N2 racemization reaction.

Aniline Trapping Experiments—To investigate further the reaction mechanism for cyclization, aniline was used to trap the carbocation intermediate. Aniline was able to stop almost completely the formation of 2 when added at concentrations of 5.65×10^{-3} M or higher to a 5.65×10^{-4} M ibutilide solution (Figure 6). The data in Figure 6 were fit with a model identical to eqs 9–11, except that a second-order rate constant for the reaction of aniline with the carbocation intermediate was added and k_{QI} was assumed to be zero. At an aniline concentration of 5.65×10^{-3} M, the initial rate of formation for the aniline–ibutilide conjugate, 1.99 (0.10) $\times$ 10^{-5} $M^{-1} \cdot day^{-1}$ (95% CI), is within 86% of the initial rate for

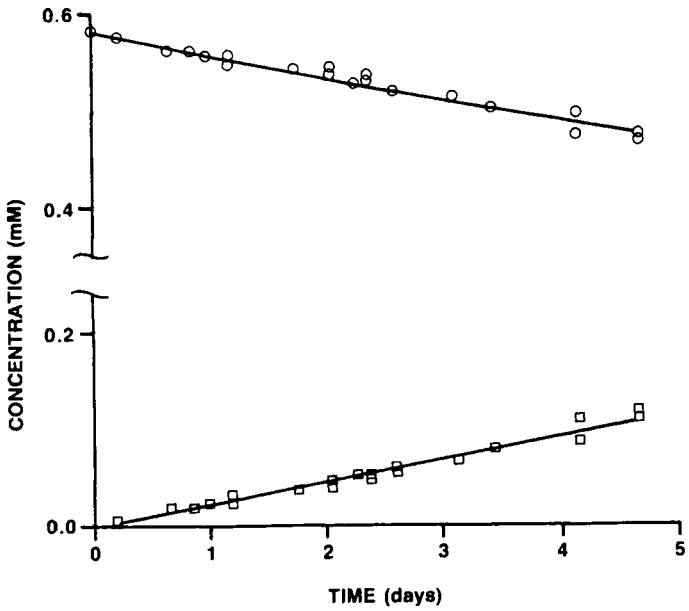


Figure 5—Concentrations of ibutilide (○) and the cyclic quaternary ammonium product (□) as a function of time at pH 4.6 and 80 °C. The lines represent the fit of the experimental data to eqs 9–11.

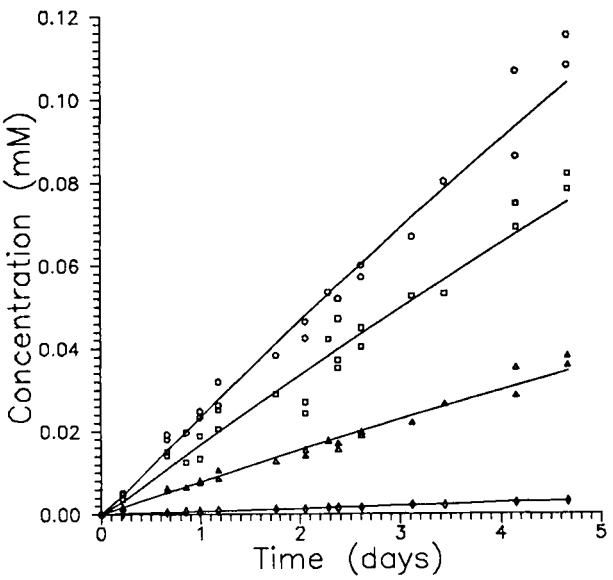


Figure 6—Effect of aniline on the rate of formation of the cyclic quaternary ammonium product from a 5.65×10^{-4} M ibutilide solution at 80 °C and pH 4.6. Key to aniline concentration (M): (○) 0; (□) 5.65×10^{-5}; (△) 5.65×10^{-4}; (◇) 5.65×10^{-3}. The lines represent the theoretical fit as described in the text.

formation of quaternary ammonium product without aniline. These results provide further evidence that the cyclization reaction proceeds primarily through an S_N1-type mechanism.

The effect of aniline on racemization kinetics was also investigated. Figure 7 shows the formation of the dextrorotatory enantiomer of ibutilide from a solution of the levorotatory enantiomer in the absence and presence of 10-fold excess aniline. If, like cyclization, racemization proceeds solely through the carbocation intermediate, then the rate of racemization would be expected to drop to nearly zero under these conditions. However, the fact that aniline decreases the

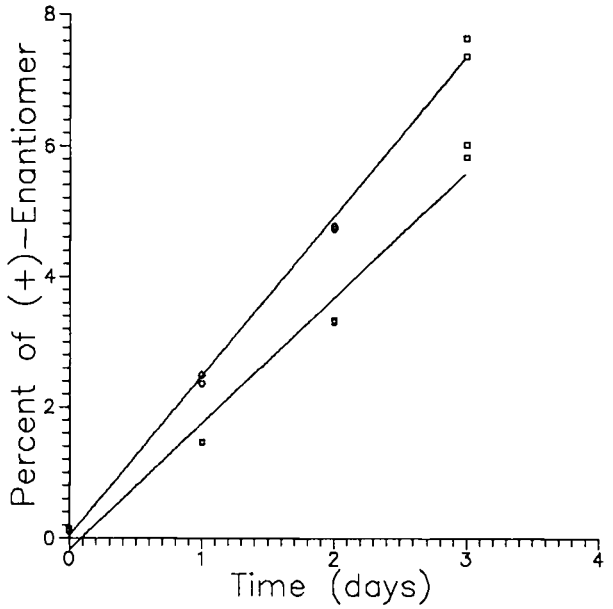


Figure 7—Formation of the dextrorotatory enantiomer of ibutilide from a 5.65×10^{-4} M solution of the levorotatory enantiomer at pH 4.6 and 80 °C. Key: (○) control; (□) 5.65×10^{-3} M aniline.

rate of racemization only by ~21% suggests that a direct S_N2 mechanism is the primary mechanism for racemization. It is possible that an S_N2-type mechanism is not observed for cyclization because of the excessive steric hindrance of the tertiary amine. Steric hindrance is not expected to be as important a factor for the attack of water at the chiral carbon.

Conclusions

The racemization and cyclization reactions of ibutilide are similar in that both involve nucleophilic attack at the chiral carbon. On the basis of the bell-shaped pH–rate profile, kinetic analysis, aniline trapping of the carbocation intermediate, and observation of all four stereoisomers of **2**, cyclization appears to proceed via a carbocation intermediate. Whereas racemization can also progress through the same intermediate, a direct S_N2 mechanism appears to be the major route for the racemization reaction.

References and Notes

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4. Actually, **2** has four stereoisomers because it has two chiral centers.
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Acknowledgments

We thank P. J. DeMulder and G. E. Amidon for their assistance with the determination of the acid dissociation constants, L. D. Kissinger for his discussions on the cyclization reaction, and T. J. Vidmar for his assistance with the nonlinear fitting of data.