Kinetics of Procaterol Auto-oxidation in Buffered Acid Solutions

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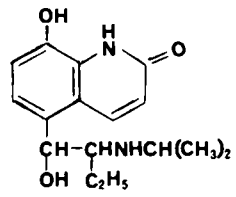
Abstract □ The kinetics of procaterol (**1**) degradation in buffered acidic solutions (pH 4–6) was investigated using an HPLC procedure. The effect of temperature and ferric ions on the reaction rate was estimated. In acidic solutions, **1** undergoes pseudo first-order degradation with an induction period. The first-order rate constant for degradation increased and the induction period decreased with an increase in pH. Ferric ions catalyzed the degradation reaction and decreased the induction period. At pH 6, the activation energy of the reaction was 34.5 kcal/mol/deg. The results of this study indicate that **1** in solution is more stable at acidic pH, in the absence of heavy metal ions, and protected from air.

Procaterol [(±)-*erythro*-8-hydroxy-5-[1-hydroxy-2-(isopropylamino)-butyl]carbostyril; **1**], a new sympathomimetic amine with potent bronchodilatory activity, high β-selectivity, and prolonged effectiveness,[1] has shown capricious patterns of oxidative decomposition in pharmaceutical preparations. Some samples of tablets have shown satisfactory stability for the first 6–12 months of storage, followed by a drop in procaterol assay and an increase in oxidation product, 5-formyl-8-hydroxycarbostyril. Tablets packaged singly in 2-dram flint vials (~7 mL) with plastic screw caps showed a greater extent of degradation than tablets packaged in well-filled high-density polyethylene bottles. Furthermore, an aqueous solution of procaterol stored in sealed ampuls at 37 °C for 18 d showed a 67.8% degradation as compared with only 11.2% degradation when the air in the head space of the ampul was replaced by nitrogen. These observations led to the conclusion that the side chain of procaterol is susceptible to cleavage via oxidation by molecular oxygen. The purpose of the present investigation was to study the kinetics of oxidative degradation of procaterol in buffered acid solutions with and without the presence of heavy metal ions. The study was initiated to determine those parameters that will be of value in preparing stable dosage forms.



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Experimental Section

Materials—All chemicals used in this study were reagent grade. Procaterol hydrochloride hemihydrate (purity 99.5%) and its threo isomer, 5-formyl-8-hydroxycarbostyril, and 8-hydroxycarbostyril were supplied by Otsuka Pharmaceutical Company, Japan.

Analytical Procedure—The high-pressure liquid chromatograph was equipped with a pump (Dupont 870), an autosampler (Perkin-Elmer LC-420) with a precise 20-μL loop injector, a detector (Beckman 160 Absorbance Detector), and a computing integrator (Spectra-Physics SP-4100). The separation was carried out using a 15 cm × 4.6 mm octadecylsilane column (Altex Ultrasphere ODS column) with a mobile phase consisting of 800 vol of a solution of 0.005 M sodium 1-heptane-sulfonate (Eastman Kodak Company, Rochester, NY) in 2% acetic acid, 150 vol of methanol (Omni Solv, glass distilled, EM Science, EM Industries, Inc., Gibbstown, NJ), and 100 vol of acetonitrile (Omni Solv, glass distilled, EM Science, EM Industries, Inc., Gibbstown, NJ). A flow rate of 1.7 mL/min was used with the UV detector fixed at 254 nm. The detector sensitivity was set at 0.1 AUFs and the integrator attenuation was set at 8. A standard solution containing ~8 μg/mL of procaterol hydrochloride hemihydrate, 2 μg/mL of 5-formyl-8-hydroxycarbostyril, 1 μg/mL of 8-hydroxycarbostyril, and 1 μg/mL of the threo isomer was prepared in the mobile phase. A 25-fold dilution of each sample with the mobile phase was made.

Solutions—A procaterol stock solution containing ~1 mg/mL of procaterol hydrochloride hemihydrate was prepared in distilled water. The sodium acetate stock solution consisted of 41 mg/mL of anhydrous sodium acetate in distilled water. The sodium phosphate, monobasic stock solution consisted of 60 mg/mL of sodium phosphate monobasic in distilled water. The ferric chloride hexahydrate stock solution contained ~1.2 mg/mL of ferric chloride hexahydrate in distilled water. A stock solution of 0.63 mg/mL of cupric acetate monohydrate was prepared in distilled water.

To prepare solutions of procaterol in 0.1 M acetate buffer, 20-mL aliquots of sodium acetate stock solution and 20 mL of procaterol stock solution were pipetted into 100-mL volumetric flasks containing ~50 mL of distilled water. The solutions were adjusted to pH 4.0 ± 0.1, 4.5 ± 0.1, 5.0 ± 0.1, and 5.5 ± 0.1 by the dropwise addition of glacial acetic acid, then diluted to the mark with distilled water.

To prepare solutions of procaterol with ferric ion in 0.1 M acetate buffer, 20-mL aliquots of procaterol stock solution, 20 mL of sodium acetate stock solution, 1 mL of ferric chloride hexahydrate stock solution, and ~50 mL of distilled water were added to 100-mL volumetric flasks. The solutions were adjusted to pH 4.0 ± 0.1, 4.5 ± 0.1, 5.0 ± 0.1, and 5.5 ± 0.1 by the dropwise addition of glacial acetic acid, then diluted to the mark with distilled water.

The solutions of procaterol, buffered at pH 6.0, were prepared by the following method. Aliquots (25 mL) of sodium acetate stock solution, 25 mL of sodium phosphate monobasic stock solution, 50 mL of procaterol stock solution, and ~100 mL of distilled water were mixed in a 250-mL volumetric flask. The solution was adjusted to pH 6.0 ± 0.1 with 1 M sodium hydroxide and diluted to the mark.

Procaterol solution containing 2 μg/mL of ferric ion was prepared by mixing 10-mL aliquots of sodium acetate stock solution, 10 mL of sodium phosphate, monobasic stock solution, 20 mL of procaterol stock solution, 1 mL of ferric chloride hexahydrate stock solution, and ~50 mL of distilled water in a 100-mL volumetric flask. The solution was adjusted to pH 6.0 ± 0.1 with 1 M sodium hydroxide solution and diluted to the mark. Procaterol solution containing 20 μg/mL of ferric ion was prepared by mixing 10-mL aliquots of sodium acetate stock solution, 10 mL of sodium phosphate monobasic stock solution, 20 mL of procaterol stock solution, 10 mL of ferric chloride hexahydrate stock solution, and ~40 mL of distilled water in a 100-mL volumetric flask. The solution was adjusted to pH 6.0 ± 0.1 with 1 M sodium hydroxide solution and diluted to the mark.

Procaterol with copper ion, buffered at pH 6.0, was prepared by mixing 10-mL aliquots of sodium acetate stock solution, 10 mL of sodium phosphate monobasic stock solution, 20 mL of procaterol

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stock solution, 1 mL of cupric acetate stock solution, and ~50 mL of distilled water in a 100-mL volumetric flask. The solution was adjusted to pH 6.0 ± 0.1 with 1 M sodium hydroxide solution and diluted to the mark.

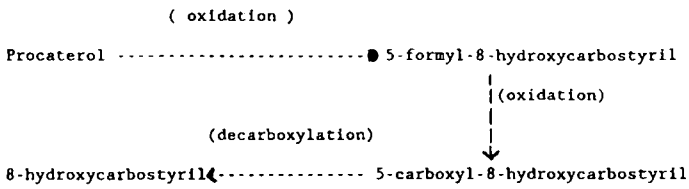
Effect of pH on Reaction Rate—Ten 5-mL aliquots of procaterol solutions buffered at each of the pH values, with or without ferric or cupric ion, were transferred to 10-mL ampuls, and the ampul tips were heat sealed. One ampul from each set was refrigerated at 4 °C to serve as the "zero-time", and the remaining nine ampuls in each set were immersed in a constant temperature bath (Ex-500 model, Neslab Instrument, Inc., Portsmouth, NH) set at 80 ± 0.1 °C. Ampuls were removed from the bath at appropriate intervals, cooled, and stored refrigerated at 4 °C for HPLC analysis.

Effect of Temperature on Reaction Rate—Five-milliliter aliquots of the solution buffered at pH 6.0 were transferred to each of 41 10-mL ampuls. The sealed ampuls, excluding the one which was retained at 4 °C as the "zero-time" sample, were divided into sets of 10. Each set of ampuls was heated in constant-temperature baths at 60, 70, 75, and 80 °C. Ampuls were removed from each bath at appropriate intervals, cooled, and stored refrigerated at 4 °C for HPLC analysis.

Results and Discussion

Ion-Paired High-Performance Liquid Chromatographic Assay of Procaterol—The HPLC mobile phase of 2% acetic acid:methanol:acetonitrile with 0.005 M sodium 1-heptanesulfonate (detector wavelength fixed at 254 nm and a flow rate of 1.7 mL/min) provided a chromatogram (Figure 1) with a steady baseline and the specificity and sensitivity required for quantitative analyses of procaterol. The retention times for procaterol and its degradation products, 5-formyl-8-hydroxycarbostyril, 8-hydroxycarbostyril, and the threo isomer, are 7.5, 3.5, 4.0, and 8.5 min, respectively. Linearity was observed for procaterol in the region of 2 to 10 μg/mL when peak height responses were plotted versus concentrations, and the line passed through the origin when extrapolated to zero concentration.

Kinetics—Procaterol undergoes two possible degradation routes: isomerization and oxidation. However, isomerization occurs only at refluxing temperature in 0.1 M hydrochloric acid solution. In the temperature and pH ranges used in the experiment, procaterol degraded only through the oxidation reaction following the reaction scheme:



Scheme

5-Carboxyl-8-hydroxycarbostyril is unstable; therefore, only 5-formyl-8-hydroxycarbostyril and/or 8-hydroxycarbostyril would be found during the course of the oxidation reaction. The rate studies carried out in the experiment followed the rate of disappearance of the intact procaterol.

Effect of pH on Reaction Rate—The regression lines for the graph of $\ln C_o/C_t$ versus time are shown in Figure 2 (in the absence of ferric ion) and in Figure 3 (in the presence of ferric ion). The graphs fit the first-order rate equation with

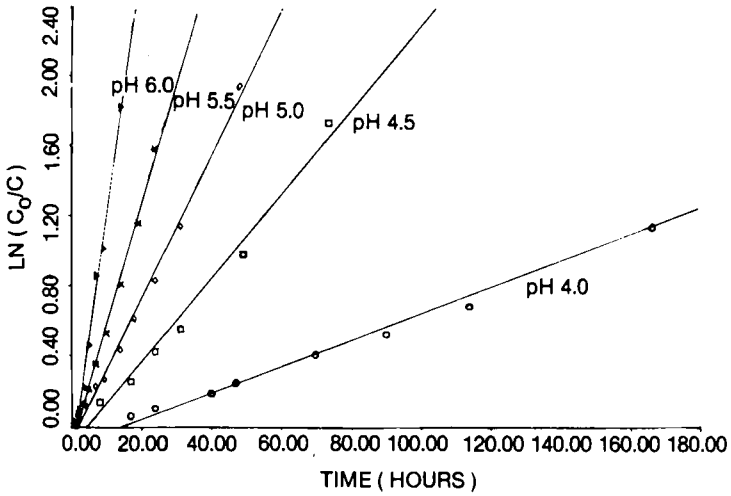


Figure 2—*First-order plots of procaterol decomposition at 80 °C.*

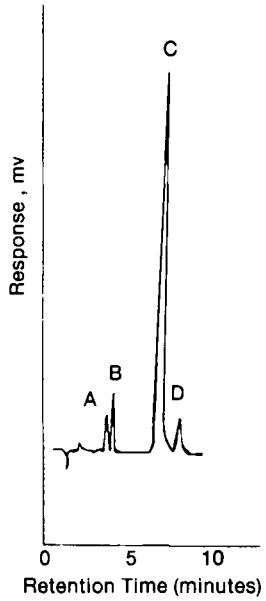


Figure 1—*Chromatogram of procaterol and its degradation products. (A) 5-Formyl-8-hydroxycarbostyril; (B) 8-hydroxycarbostyril; (C) procaterol; (D) threo isomer.*

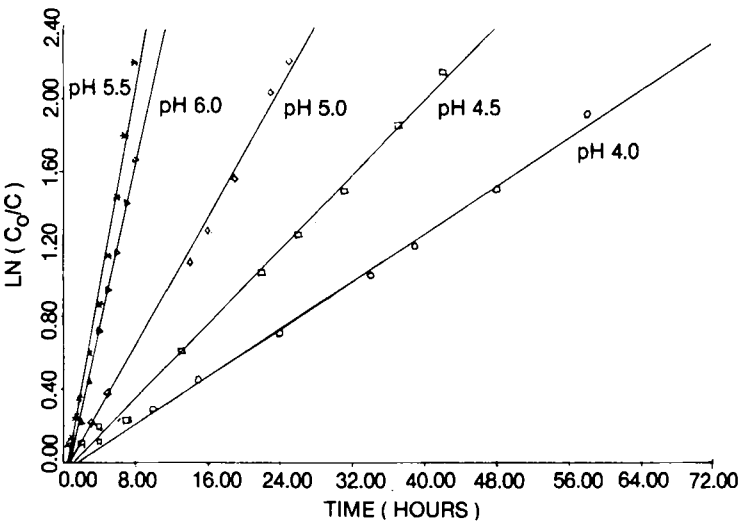


Figure 3—*First-order plots of procaterol decomposition at 80 °C, in the presence of ferric ion.*

correlation factors of 0.99694, 0.99310, 0.99712, 0.99809, and 0.99630 obtained from solutions (in the absence of ferric ion) of pH 4.0, 4.5, 5.0, 5.5, and 6.0, respectively. In the presence of ferric ion, correlation factors of 0.99844, 0.99839, 0.99761, 0.99446, and 0.99894 were obtained from solutions of pH 4.0, 4.5, 5.0, 5.5, and 6.0, respectively. A 10-mL ampul has a total volume of ~14-mL after sealing, so that the 9 mL of headspace contains $\sim 75 \times 10^{-6}$ mol of oxygen compared with $\sim 3 \times 10^{-6}$ mol of procaterol in the 5 mL of solution. The 25-fold excess of oxygen accounts for the pseudo first-order kinetics observed. The best fit of the data using the regression line intercepts the time axis of the graph at points corresponding to ~2–14 h for samples in the absence of ferric ion and at points corresponding to ~0.6–1.6 h for samples in the presence of ferric ion. In both cases, a reasonable fit of the data to a regression line forced through the origin could be obtained. However, the interpretation that an induction period exists is consistent with auto-oxidation processes in general and with our observations on procaterol formulations in stability studies. The first-order rate constants, the half-lives, and the induction periods calculated from these data are shown in Table I. Figure 4 shows pH–rate profiles for the auto-oxidation of procaterol with and without the presence of iron. Both lines run in parallel; the rate of auto-oxidation of procaterol in both cases increases inversely with the concentration of hydrogen ion in the medium.

Table I—Kinetic Parameters of Procaterol Degradation in Acid Buffered pH at 80 °C

pH	$k \times 10^2$, h^{-1}		$t_{1/2}$, h		I, h	
	Fe^{+++}	Fe^{+++}	Fe^{+++}	Fe^{+++}	Fe^{+++}	Fe^{+++}
	Present	Absent	Present	Absent	Present	Absent
4.0	3.3	0.76	21.0	91.2	1.6	14.3
4.5	5.1	2.4	13.5	28.9	1.1	4.6
5.0	8.9	4.1	7.8	16.9	0.7	2.2
5.5	28.0	6.9	2.5	10.0	0.6	1.7
6.0	22.2	20.1	3.1	3.5	1.6	2.0

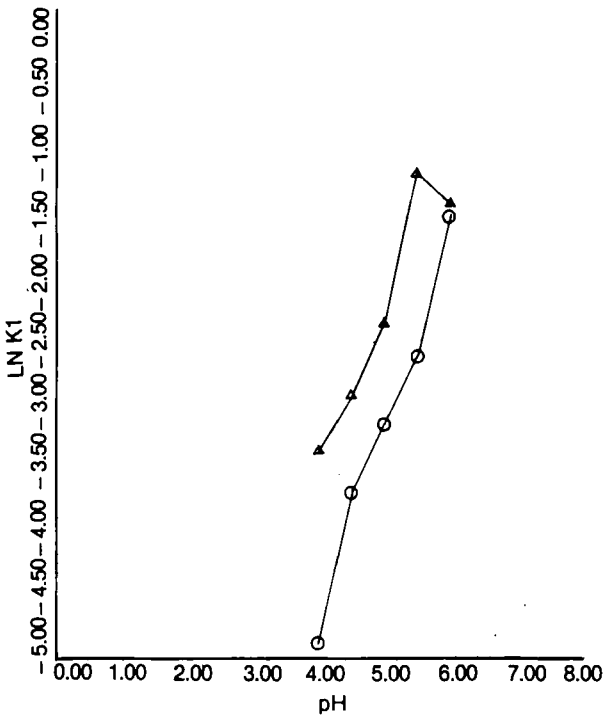


Figure 4—*The pH–rate profile for procaterol oxidation. Key: (△) in the presence of Fe^{+++} (2 μg/mL), (○) in the absence of Fe^{+++}.*

With the exception of solutions at pH 6.0, where a mixed buffer of acetate and phosphate was used, catalytic action by ferric ion resulted in a 2–4-times increase in the auto-oxidation rate of procaterol and simultaneously greatly diminished the duration of its induction period. Phosphate in the mixed buffer, being a good metal-complexing agent, may bind the trace quantity of ferric ion. Thus, the metal becomes unavailable for catalytic action. This speculation is supported by the observation that a 10-fold increase of ferric ion concentration to 20 μg/mL resulted in precipitation of ferric phosphate with a marginal increase in reaction rate (k_1 = 0.236 h^{-1}). However, replacing iron with cupric ion (2 μg/mL) yielded a 40% gain in observed pseudo first-order rate constant to 0.285 h^{-1} and an induction period of ~0.5 h.

Effect of Temperature on Reaction Rate—Figure 5 shows the pseudo first-order kinetics for the pH 6.0 solution without the presence of ferric ion at the four temperatures selected. Correlation factors of 0.99930, 0.99876, 0.99553, and 0.99630 were obtained at temperatures of 60, 70, 75, and 80 °C, respectively. The observed rate constants are presented in Table II. Each of the regression lines plotted in Figure 5 shows an induction period, the length of which is inversely proportional to temperature. As noted earlier, these plots could have been forced through the origin. The Arrhenius plot of log k_1 versus $1/T$ is shown in Figure 6. The activation energy calculated by the equation $E_a = -2.303 \times 1.987 \times$ (slope of the line), is 34.5 Kcal/mol/deg, where the slope of the line is −7543 K. This energy of activation includes a term for the variation of the oxygen solubility with temperature. The decreased solubility of oxygen with increasing temperature is partially compensated for by the increased solubility due to increased oxygen pressure above the solution as the temperature is raised. Extrapolation of the plot in Figure 6 to 25 °C gives an estimate for the rate constant at nominal room temperature of 0.0000209 h^{-1}. At pH 6.0, then, a 10% decomposition would occur after 5041 h or ~7 months. Since the oxidation of procaterol involves an induction period, the

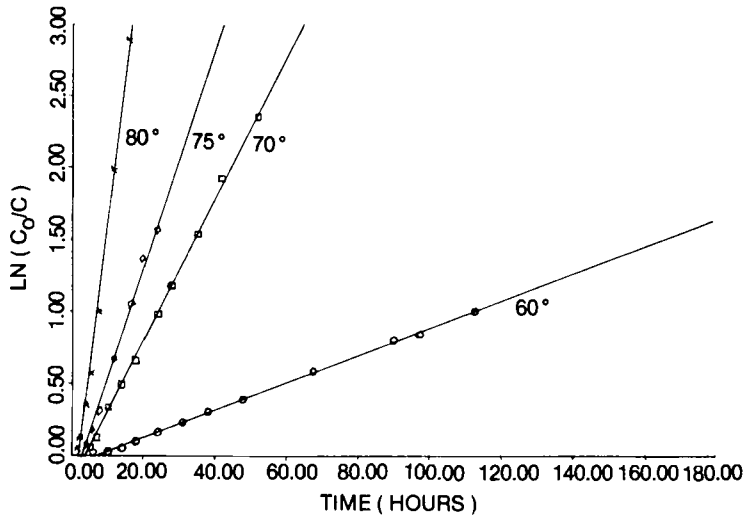


Figure 5—*Effect of temperature on procaterol oxidation at pH 6.0.*

Table II—Rate Constants for Procaterol Solution at pH 6.0

Temperature, °C	k_1, h^{-1}
60	0.0095
70	0.0486
75	0.0755
80	0.2008

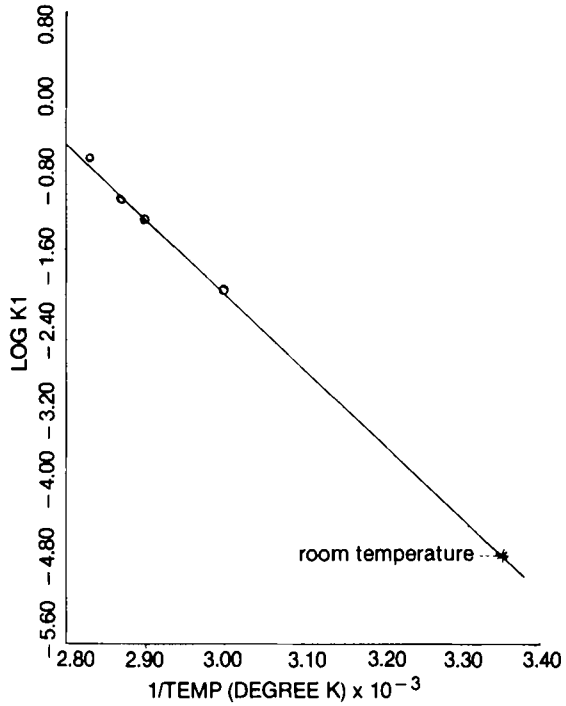


Figure 6—*Arrhenius plot of procaterol oxidation at pH 6.0.*

actual shelf-life would be >7 months, depending on the length of induction period at room temperature.

Induction Period—The induction period inferred from the data is believed to be real. It is interpreted as the time during which a sufficient concentration of the free radicals is built up to propagate the reaction. The kinetic data measure the reaction during the propagation phase. The length of the induction period varies directly with the stability of the compound under the conditions studied.

Conclusions

Manufacturing conditions should be designed to minimize exposure to oxygen (air) and provide as acidic an environment as practical. Pharmaceutical excipients used in liquid formulations should be tested for iron and other heavy metals to assure they do not exceed the limit which will cause excessive degradation.

References and Notes

1. Yoahizaki, Shiro; Manobe, Yoahiaki; Tamada, Snigeharu; Nakagawa, Kazuyuki; Tei, Seiso. *J. Med. Chem.* **1977**, *20*, 1103.