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Degradative Behavior of a New Bronchodilator, Carbuterol, in Aqueous Solution

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Abstract □ The degradation kinetics of carbuterol in aqueous solution were investigated at 85° and constant ionic strength over the pH 0.25–13.3 range under anaerobic conditions. The results demonstrated a complex kinetic pattern involving specific acid and specific base catalyses at the pH extremes. Degradation resulted primarily from intramolecular catalysis and indicated that both the protonated and unprotonated phenolic groups participated in the reaction. High-pressure liquid chromatography was used to isolate carbuterol and its degradation product. Mass spectrometric examination showed that the degradation product was a cyclized derivative formed by intramolecular attack of the phenoxy group on the ureido carbonyl with ammonia expulsion. The apparent activation energy for carbuterol at pH 4.0 and 10.0 was 22.3 and 11.7 kcal/mole, respectively. The agreement between the calculated theoretical pH–rate profile and the experimental points supports the hypothesis presented concerning the reactions involved in carbuterol degradation.

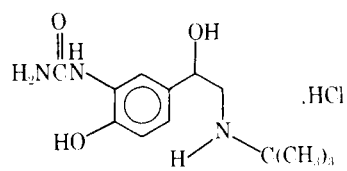
Keyphrases □ Carbuterol—degradation kinetics in aqueous solution, pH effect □ Degradation kinetics—carbuterol in aqueous solution, pH effect □ Kinetics—carbuterol degradation in aqueous solution, pH effect □ Bronchodilators—carbuterol, degradation kinetics in aqueous solution, pH effect

Carbuterol, [5-[2-[(1,1-dimethylethyl)amino]-1-hydroxyethyl]-2-hydroxyphenyl]urea hydrochloride (I), is a new potent bronchodilator. It is stable in solid admixtures, capsules, and tablets. Like other bronchodilators of similar structure, carbuterol oxidizes readily in aqueous solution (1, 2). Unlike other similar bronchodilators, carbuterol exhibits significant solution degradation under anaerobic conditions in the presence of antioxidants. This study investigated the unusual degradative behavior of carbuterol in aqueous solution.

EXPERIMENTAL

Materials—All chemicals were analytical reagent grade.

Buffers—Hydrochloric acid–potassium chloride (pH 0.25–2.0), citrate buffer (pH 3.0–5.5), succinate buffer (pH 6.0), phosphate buffer (pH 6.5–8.0), borate buffer (pH 8.5–9.0), carbonate buffer (pH 9.35–11.0), trisodium phosphate buffer (pH 12.0), and sodium hydroxide–potassium chloride (pH 13.0–13.3) were used. The buffers were freshly prepared by dissolving hydrochloric acid, citric acid, succinic acid, monobasic sodium phosphate, boric acid, sodium bicarbonate, tribasic sodium phosphate, or sodium hydroxide together with potassium chloride in distilled water and adding concentrated sodium hydroxide to achieve the desired pH. The buffers were 0.1 *M* with respect to hydrochloride, citrate, succinate, phosphate, borate, carbonate, and hydroxide ions (except when buffer effects were investigated) and were adjusted to an ionic strength of 0.5 with potassium chloride (except when the primary salt effect was investigated).



I

High-Pressure Liquid Chromatographic (HPLC) Isolation of Degradation Product—The liquid chromatograph[1] was fitted with a precision photometer at 254 nm. The column, 2 mm i.d. × 1 mm, was packed with strong cationic exchange[2] resin and was operated at ambient temperature, a pressure of 900 psig, and a flow rate of 1 ml/min.

The mobile phase was 0.01 *M* borate buffer containing 0.1 *M* anhydrous sodium sulfate adjusted to pH 10.0 with sodium hydroxide. The concentration was normal at about 2.5 mg/ml; the injection size was 5 μl in aqueous solution. The attenuation was 64×10^{-2}, and the chart speed was 2.54 cm/5 min.

Several injections of degraded solution were placed on the column, and the decomposition product was collected as it was eluted. The solution was evaporated to dryness, and the residue was washed several times with aliquots of methanol. The methanol subsequently was evaporated to dryness, and the residue was submitted for analysis by mass spectrometry.

[1] Dupont 820.
[2] Dupont 820960002.

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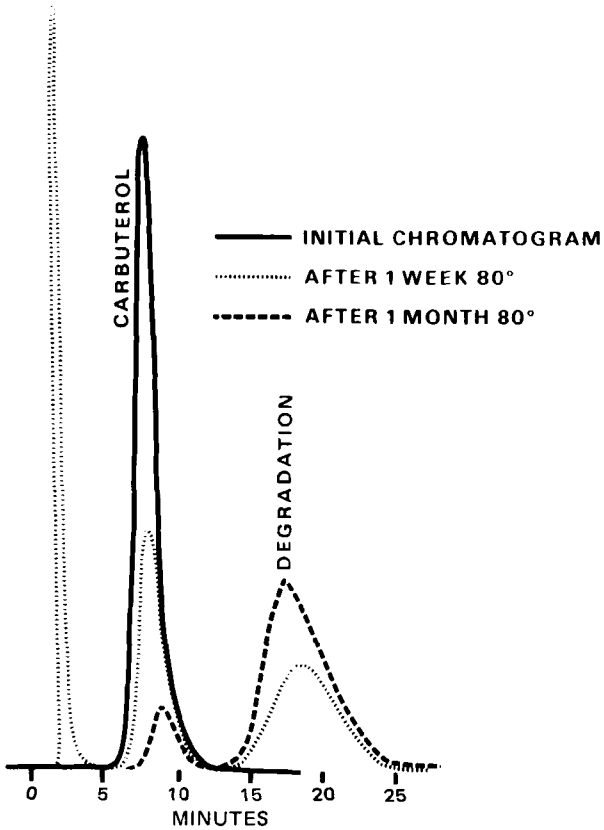


Figure 1—*HPLC separation of carbuterol from its degradation product at pH 4.0.*

Colorimetric Assay—A sensitive 4-aminoantipyrine method for phenolic compounds was used for the colorimetric determination of residual carbuterol (3).

The following reagents were added, in order, to a 100-ml volumetric flask: 50 ml of 0.4 *M* carbonate buffer (pH 10.0), 5 ml of carbuterol stock solution (0.5 mg/ml), 10 ml of 3% aqueous aminoantipyrine, and 15 ml of 4% aqueous potassium ferricyanide. The flask was brought to volume with the carbonate buffer. A reagent blank also was prepared. After

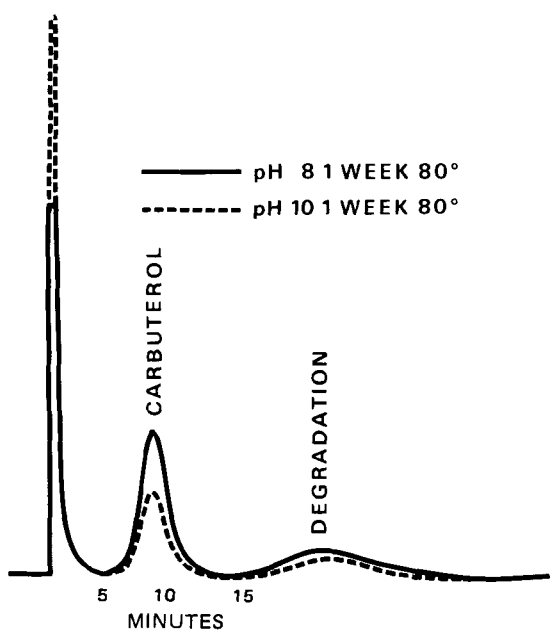


Figure 2—*Separation of carbuterol from its degradation product at pH 8.0 and 10.0.*

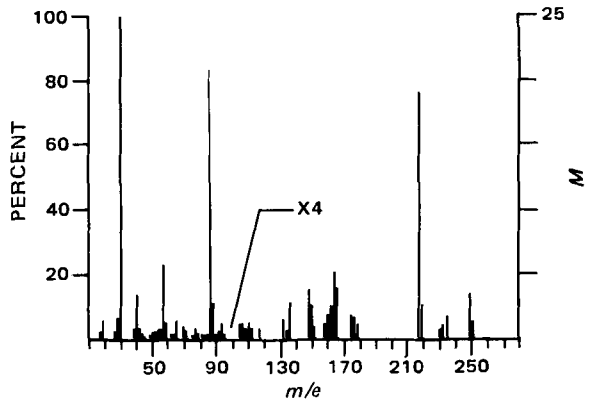


Figure 3—*Mass spectrum of carbuterol degradation product.*

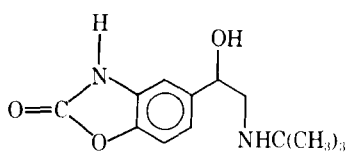
standing for 10 min to allow color development, both solutions were analyzed spectrophotometrically at 520 nm in 1-cm cells.

Kinetic Procedure—Approximately 50 mg of carbuterol was weighed accurately and dissolved in 100 ml of the appropriate buffer. The resulting solution was flushed with nitrogen and subsequently filled into 15-ml glass ampuls. The ampuls were flushed with nitrogen, sealed, and placed in a constant-temperature oil bath set at 85°. Samples were taken at various intervals and assayed using the colorimetric procedure previously described.

RESULTS AND DISCUSSION

Characterization of Degradation Product—Figures 1 and 2, typical chromatograms, indicate that the degradation product can be separated from the parent compound. The compound isolated during the kinetic studies was subjected to spectral analysis (Fig. 3). This spectrum supports a structure with a molecular ion peak at *m/e* 250 and major fragmentation peaks at *m/e* 235, 232, 217, 164, 86, 57, 41, and 30.

This product was subsequently synthesized by an unambiguous route; it was also characterized by conventional analytical, spectral, and chromatographic methods[3]. The synthetic compound gave a mass spectrum identical with that observed for the degradation product (II).



II

Reaction Order—The disappearance rate of carbuterol from aqueous solution was an overall pseudo-first-order reaction with respect to carbuterol at pH 0.25–13.0 and constant temperature and ionic strength. The relationship between time and the logarithm of the residual carbuterol concentration was linear (Fig. 4). The observed rate constants and the buffer systems are listed in Table I.

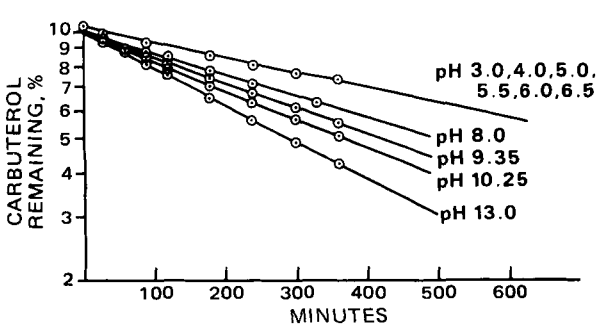


Figure 4—*Effect of pH on carbuterol degradation at 85° (μ = 0.5).*

[3] Information obtained through Dr. Carl Kaiser, Smith Kline and French Laboratories, Philadelphia, PA 19101.

Table I—Buffer Systems and Observed Rate Constants for Carbuterol Degradation at 85°

pH	Buffer System	k_{obs}, $min^{-1} \times 10^3$	k_0, $min^{-1} \times 10^3$
0.25	Hydrochloric acid	1.72	—
0.50	Hydrochloric acid–potassium chloride	1.359	—
1.0	Hydrochloric acid–potassium chloride	1.307	—
1.3	Hydrochloric acid–potassium chloride	1.195	—
2.0	Hydrochloric acid–potassium chloride	1.026	—
3.0	Citrate	0.969	—
4.0	Citrate	0.969	—
5.0	Citrate	0.976	—
5.5	Citrate	0.99	—
6.0	Succinate	0.962	—
6.5	Phosphate	1.04	—
7.0	Phosphate	1.127	1.05
7.5	Phosphate	1.233	0.972
8.0	Phosphate	1.428	1.045
8.5	Borate	1.215	—
9.0	Borate	1.52	—
9.35	Carbonate	1.66	—
10.0	Carbonate	1.925	—
10.25	Carbonate	2.05	—
10.5	Carbonate	2.09	—
10.61	Carbonate	2.10	—
11.0	Carbonate	2.068	—
12.0	Trisodium phosphate	2.27	—
13.0	Sodium hydroxide–potassium chloride	2.475	—
13.3	Sodium hydroxide	2.66	—

Catalytic Effects of Buffer Systems—The catalytic effect of the buffer systems used in the kinetic studies was determined at constant pH, temperature, ionic strength ($\mu = 0.5$), and carbuterol concentration; only the buffer concentration was varied. The citrate, borate, and carbonate buffer species did not appreciably affect carbuterol degradation. Figure 5 shows the phosphate buffer effect between pH 7.0 and 8.0. Within this pH range, only the mono- and dihydrogen phosphate ions are important. The observed rate constant is, therefore, a summation of the catalytic rate constants of the phosphate buffer species and the rate at zero buffer concentration. It may be expressed as:

$$k_{obs} = k_0 + k_{H_2PO_4^-}[H_2PO_4^-] + k_{HPO_4^{2-}}[HPO_4^{2-}] \quad \text{(Eq. 1)}$$

where k_0 is the rate constant at zero buffer concentration; the other rate constants are the catalytic rate constants due to the phosphate buffer species. By using the conservation equation and the acidity constant expression for the phosphate buffer system:

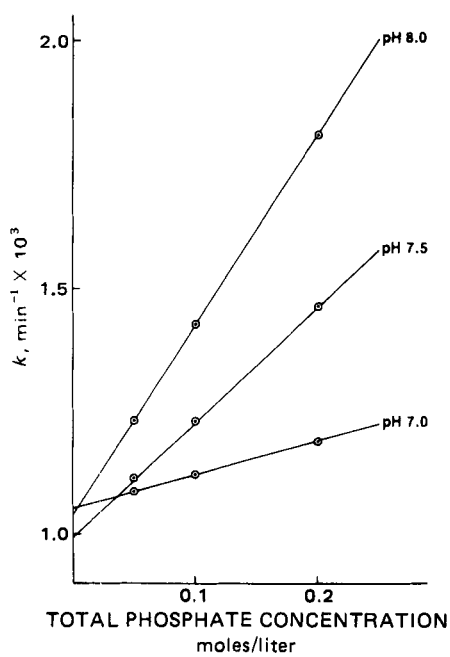


Figure 5—*Phosphate buffer catalytic effect on observed rate constants of carbuterol degradation at constant pH and 85° ($\mu = 0.5$).*

Table II—Slopes (S_{exp}) of the Lines in Fig. 5 and Slopes (S_{calc}) Calculated

pH	$S_{exp} \times 10^4$	$S_{calc} \times 10^4$
7.0	6.8	6.8
7.5	13	13
8.0	37	27

$$C_p = [H_2PO_4^-] + [HPO_4^{2-}] \quad \text{(Eq. 2)}$$

$$K_{a2} = \frac{[H^+][HPO_4^{2-}]}{[H_2PO_4^-]} \quad \text{(Eq. 3)}$$

the overall rate may be written as:

$$k_{obs} = k_0 + C_p \frac{k_{H_2PO_4^-}[H^+] + k_{HPO_4^{2-}} K_{a2}}{[H^+] + K_{a2}} \quad \text{(Eq. 4)}$$

A plot of k_{obs} *versus* the total phosphate concentration will yield a straight line with an intercept of k_0 and a slope of:

$$\text{slope} = \frac{k_{H_2PO_4^-}[H^+] + k_{HPO_4^{2-}} K_{a2}}{[H^+] + K_{a2}} \quad \text{(Eq. 5)}$$

As can be seen from Fig. 5, the phosphate buffer catalysis increased with an increase in pH. This result suggests that the monohydrogen phosphate ion is the catalyzing species for carbuterol. From Eq. 4, the phosphate buffer catalytic rate constants for carbuterol degradation at 85° were obtained and shown to be $k_{H_2PO_4^-} = 7.9 \times 10^{-4}\ min^{-1}$ and $k_{HPO_4^{2-}} = 4.87 \times 10^{-3}\ min^{-1}$. With these rate constants, theoretical slopes that agreed well with the experimental slopes were calculated for the phosphate buffer system (Table II).

Primary Salt Effect—The primary salt effect on carbuterol degradation was studied at constant pH and temperature; only the ionic strength was varied by the addition of potassium chloride. Studies were conducted at pH 4.0 and 9.0; no primary significant salt effect was observed.

pH Dependency—The pH–rate profile (Fig. 6) shows the relationship between pH and the observed reaction rate over the pH 0.25–13.0 range. The curve suggests that the overall degradation rate for carbuterol hydrolysis represents a summation of Reactions A–E shown in Scheme I.

$$\text{A:}\quad C^+ + [H^+] \xrightarrow{k_1} \text{products}$$

$$\text{B:}\quad C^+ \xrightarrow{k_2} \text{products}$$

$$\text{C:}\quad C^{\pm} \xrightarrow{k_3} \text{products}$$

$$\text{D:}\quad C^- \xrightarrow{k_4} \text{products}$$

$$\text{E:}\quad C^- + [OH^-] \xrightarrow{k_5} \text{products}$$

Scheme I

This scheme can be represented by the following equation:

$$-\frac{dC_T}{dt} = k_1[C^+][H^+] + k_2[C^+] + k_3[C^{\pm}] + k_4[C^-] + k_5[C^-][OH^-] \quad \text{(Eq. 6)}$$

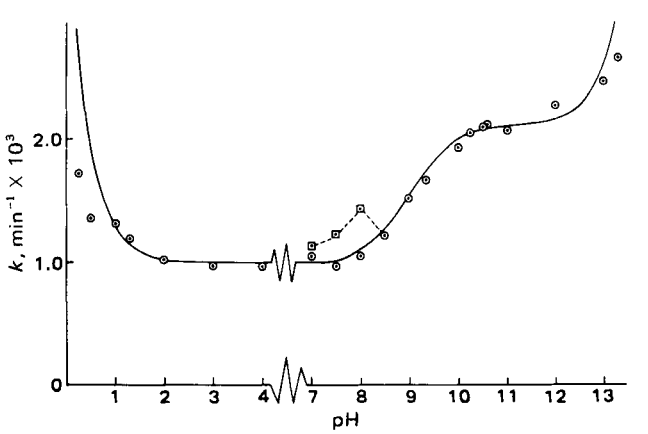
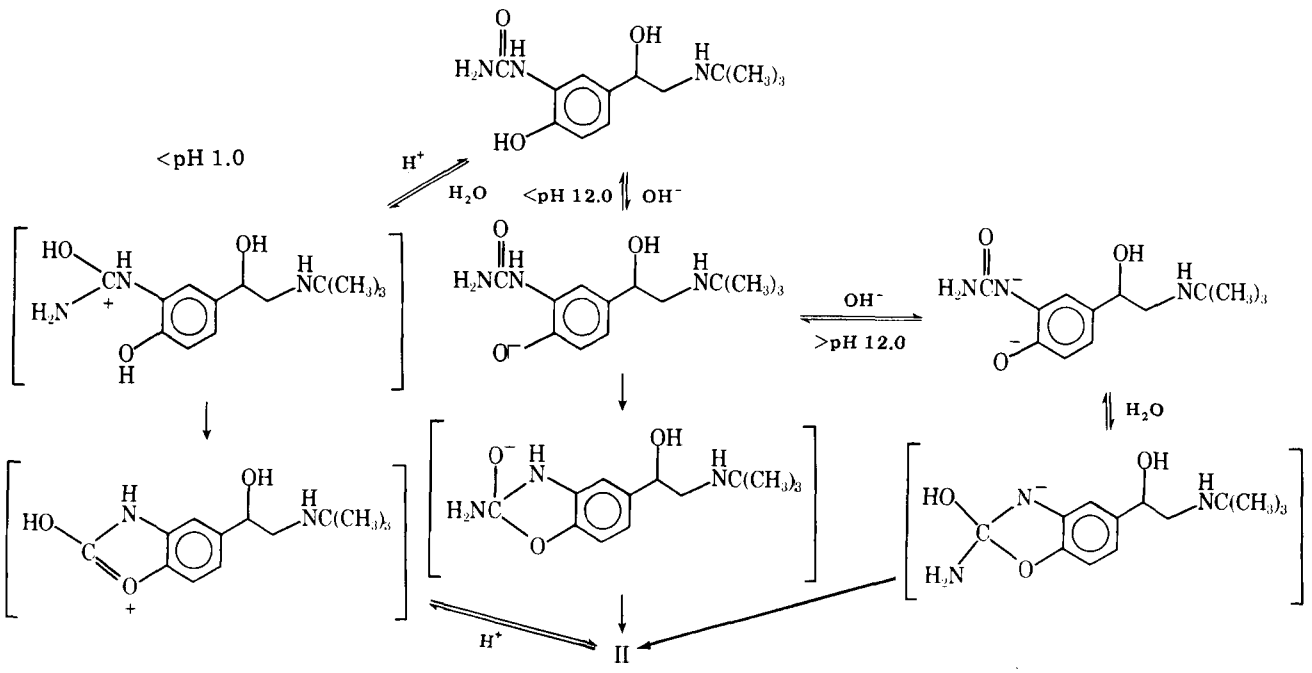


Figure 6—*The pH–rate profile of carbuterol degradation in aqueous solution at 85° ($\mu = 0.5$). Key: —, theoretical profile; ⊙, experimental profile; and ⊡, rates uncorrected for buffer effect.*



Scheme II

where:

$$C_T = [C^+] + [C^\pm] + [C^-] \quad \text{(Eq. 7)}$$

$$K_1 = \frac{[C^\pm][H^+]}{[C^+]} \quad \text{(Eq. 8)}$$

$$K_2 = \frac{[C^-][H^+]}{[C^\pm]} \quad \text{(Eq. 9)}$$

By combining these equations, the overall degradation rate can be represented by:

$$k_{obs} = \frac{k_1[H^+]^3 + k_2[H^+]^2 + k_3K_1[H^+] + k_4K_1K_2 + k_5K_1K_2K_W/[H^+]}{K_1K_2 + K_1[H^+] + [H^+]^2} \quad \text{(Eq. 10)}$$

The theoretical disappearance rate of carbuterol was calculated for the pH 0.25–13.0 range. The rate constants were approximated from the experimental data at pH intervals where the reactions in Scheme I were specifically involved. The following values provided the best fit: $k_1 = 3 \times 10^{-3}$ min^{-1}, $k_2 = 1 \times 10^{-3}$ min^{-1}, $k_3 = 2.1 \times 10^{-3}$ min^{-1}, $k_4 = 2.1 \times 10^{-3}$ min^{-1}, $k_5 = 5 \times 10^{-3}$ min^{-1}, $K_1 = 1.862 \times 10^{-8}$, $K_2 = 5 \times 10^{-12}$, and $K_W = 5.62 \times 10^{-13}$. The calculated rate (solid line in Fig. 6) is in good agreement with the experimentally determined rate.

The data indicate that below pH 1.0 the degradation rate is due to hydrogen-ion attack on the protonated species. The fact that the slope of the line below pH 1.0 is −1 suggests that Reaction A is solely responsible for the degradation. In the pH range from 1.0 to approximately 9.0, the degradation rate is essentially constant. Degradation proceeds spontaneously with water as the catalyzing species. Reaction B is responsible for this behavior.

In the pH 9.0–10.0 range, the reaction rate increases with a slope of nonintegral value. The reactions proceed spontaneously with water attack on both the protonated species and the zwitterion. These processes can be represented by Reactions B and C.

Between pH 10.0 and 12.0, the reaction rate remains constant. The reaction proceeds spontaneously with water attack on the zwitterionic species and can be represented by Reaction D.

Above pH 12.0, the reaction rate increases. The slope of the reaction rate plot is +1, suggesting that the principal reaction (E) in this range is hydroxide-ion attack on the ionized species.

Temperature Dependency—Rate constants for the overall disappearance of carbuterol were obtained from 60 to 85° at pH 4.0 and 10.0. Typical Arrhenius plots are shown in Fig. 7. From these data, the apparent activation energy at pH 4.0 and 10.0 was determined to be approximately 22.3 and 11.7 kcal/mole, respectively.

Possible Degradation Pathway—Scheme II illustrates the possible degradation pathway for carbuterol in aqueous buffered solutions. It involves the protonation or ionization of carbuterol and subsequent water attack, resulting in the appearance of II, which was isolated and characterized by mass spectral analysis. Compound II did not appear to be stable at elevated pH since the high-pressure liquid chromatograms in Fig. 2 indicated that there was no accumulation as the degradation increased.

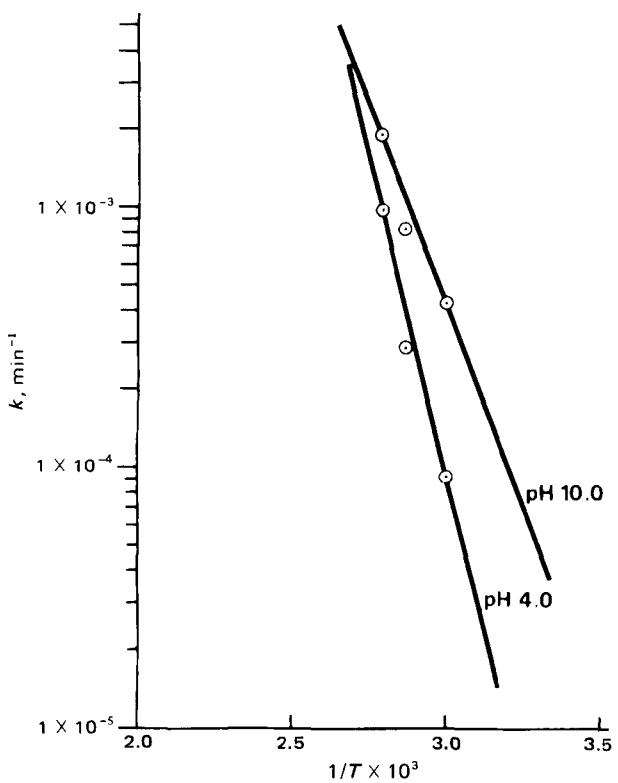


Figure 7—*Typical Arrhenius-type plot depicting the temperature dependency of carbuterol hydrolysis at pH 4.0 and 10.0.*

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