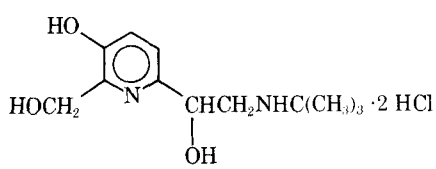
# Stability of Aqueous Solutions of Pirbuterol

P. C. BANSAL * and D. C. MONKHOUSE ˣ

**Abstract** □ The hydrolytic degradation of pirbuterol was investigated under saturated oxygen conditions over a wide range of pH values and at different temperatures. Two of the five observed breakdown products were positively identified. The first-order decomposition rate appeared to depend on the rate constants of the four dissociated ionic species. The most stable region for the drug was pH 1–2, where the diprotonated molecule predominated; appropriate thermodynamic parameters were calculated.

**Keyphrases** □ Pirbuterol—aqueous solutions, stability, effect of pH and temperature □ Stability—pirbuterol, aqueous solutions, effect of pH and temperature □ Degradation, hydrolytic—pirbuterol, effect of pH and temperature □ Bronchodilators—pirbuterol, stability of aqueous solutions, effect of pH and temperature

Pirbuterol[1] (I) is a potent sympathomimetic bronchodilator that possesses high selectivity for pulmonary as opposed to cardiac adrenergic receptors. Exploratory studies indicated that satisfactory pirbuterol stability could be attained in acidic media, while degradation occurred under alkaline conditions. Since this bronchodilator will be manufactured in many liquid dosage forms (aerosol, oral spray, intravenous injection, and pediatric syrup), its solution stability profile should be well characterized and understood.



I

The stability of pirbuterol should be superior to that of the catecholamines, since intramolecular cyclization leading to indole derivatives can be excluded on mechanistic grounds. Its stability is more likely to compare with that of the more stable compounds terbutaline and phenylephrine. Upon oxidation, these compounds produce formaldehyde, which can further react with the intact molecule to form isoquinoline derivatives *via* the Pictet–Spengler reaction (1, 2).

The purpose of this study was to evaluate pirbuterol in the light of what has already been reported about structurally similar agents of contrasting stability behavior. The stability of pirbuterol's closest analog, albuterol, has not yet been reported.

## EXPERIMENTAL

**Quantitative TLC Assay**—Pirbuterol[2] solutions (0.5–5.0 mg/ml) were spotted (5 μl) on scored precoated silica gel TLC plates[3] using capillary pipets[4]. Following each application, the pipet was rinsed with 5 μl of ethanol, which was applied to the respective spot to ensure complete transfer. Then the pipet was rinsed with the next solution to be spotted. The same pipet was used for each series. Standard samples containing known amounts of pirbuterol were spotted alternately with the unknown samples.

The chromatographic bath was prepared in a glass chamber (30 × 22 × 10 cm) by mixing 40 ml of acetic acid and 40 ml of water into 240 ml of methyl ethyl ketone. The chamber was lined on one side with filter paper, which was in contact with the mobile phase. The chamber was tightly sealed and allowed to equilibrate overnight.

After drying the applied spots thoroughly, chromatographic development was performed until the solvent front was 14–16 cm above the origin. The plates were removed from the chamber and dried in a stream of forced warm air. The reflectance of the pirbuterol spot ($R_f$ 0.43) was measured on a double-beam spectrodensitometer[5] at 282 nm, using a slit width of 1 mm and a sensitivity of 0.4. Peak areas were measured with a computing integrator[6].

**Kinetic Studies**—Aqueous 5-mg/ml solutions of pirbuterol were prepared (initial pH ~3), and the pH was adjusted with 1 *N* HCl or NaOH to integral values of pH from 1 to 14. Since preliminary investigation showed that oxygen played a significant role in the degradation of pirbuterol, the oxygen tension was kept constant in the test solutions. Oxygen saturation was achieved by bubbling oxygen through each solution throughout the experiment. The apparatus used is shown in Fig. 1. Oxygen was bubbled into the solution at a rate of 6 ml/min and was allowed to escape through a condenser, which prevented loss of water vapor.

The test solution pH was adjusted periodically by the addition of 1 *N* NaOH or HCl, utilizing a pH meter equipped with an electrode[7] specially

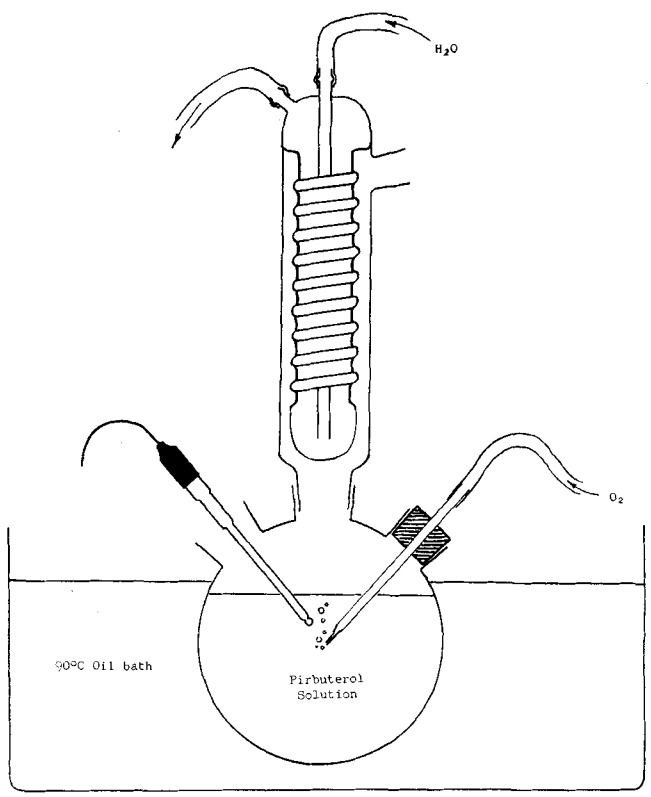



**Figure 1**—*Apparatus used for kinetic studies of pirbuterol degradation as a function of pH under stressed oxidative conditions.*

[1] α⁶-[[(1,1-Dimethylethyl)amino]methyl]-3-hydroxy-2,6-pyridinedimethanol dihydrochloride, CP-24,314-1, Pfizer Inc.
[2] Lot 8267-140-1.
[3] Silica gel GF, 20 × 20 × 0.025 cm, Uniplate, Analtech, Inc.
[4] SGA Co.
[5] Schoeffel model SD 3000.
[6] Spectra-Physics, Autolab System I.
[7] A pH meter 26 with GK2301C combined electrode, Radiometer, Copenhagen.

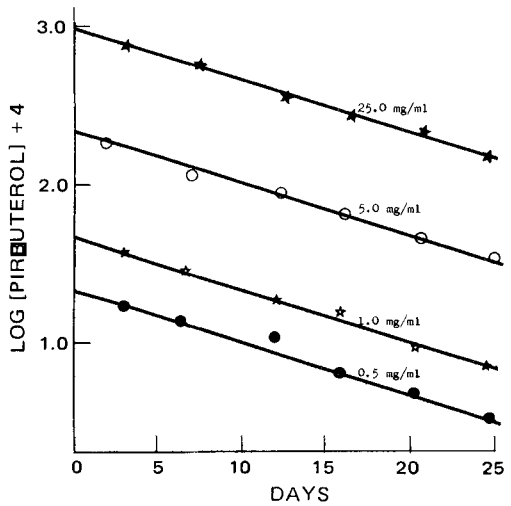



**Figure 2**—*Plot illustrating first-order dependence in pirbuterol concentration at pH 7 and 90°.*

prepared for use at high temperatures. To prevent aging and poisoning of the electrode, immersion in the actual reaction mixtures was kept to a minimum. At no time was the pH allowed to deviate more than 0.2 pH unit from the original settings. Only those solutions with pH >5 required periodic adjustment.

Temperature was controlled by immersing the solution flask into a constant-temperature oil bath. Loss of intact drug was monitored by TLC–densitometry.

**Determination of Ionization Constants**—The pKa's of pirbuterol were determined at 21 and 90°. A 0.25-mmole sample of drug was dissolved in freshly boiled double-distilled water and titrated with 1 *N* NaOH (carbon dioxide free). Change in pH as a function of the titrant delivered was monitored with a pH meter equipped with the high temperature electrode[7]. Confirmation of pKa's was achieved by titrating the structurally similar compounds 2,6-bis(hydroxymethyl)-3-hydroxypyridine and 2-(*tert*-butylamino)ethanol.

**2-Hydroxymethyl-3-hydroxypyridine-6-carboxaldehyde (II)**—An authentic sample of this compound[8] was prepared, and the structure was confirmed by NMR, IR, and elemental analyses. It exhibited an $R_f$ value of 0.75 on the TLC system previously described.

## RESULTS AND DISCUSSION

For the quantitative TLC procedure, the densitometric response for pirbuterol is linear and can be quantitated over a 0.5–5.0-mg/ml range when 5-μl spots are applied. The calibration plot (electronic peak area *versus* quantity spotted) for the assay had a correlation coefficient of 99.9% with a standard deviation of ±3.9%.

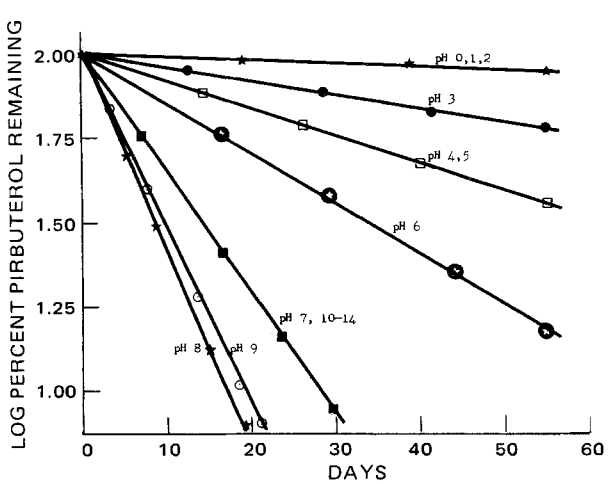



**Figure 3**—*First-order degradation of pirbuterol at 90° at various pH values.*

[8] CP-34,028, Pfizer Inc.

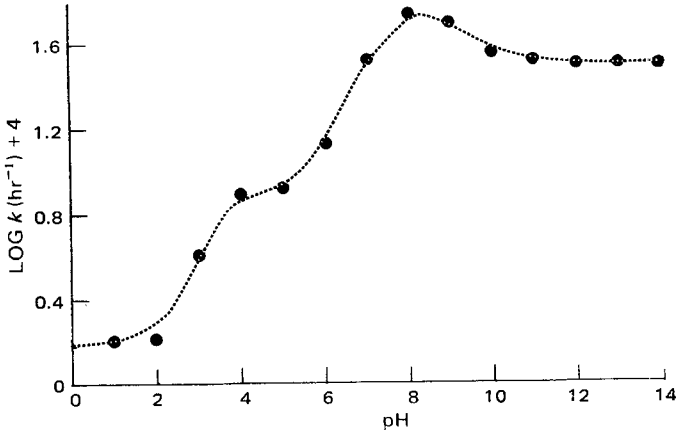



**Figure 4**—*The pH–rate profile of pirbuterol degradation at 90° as a function of pH.*

In the present study, the degradation of pirbuterol was investigated over a pH range of 1–14. These studies were conducted at 90° to accelerate the decomposition rates so that their measurement would be kinetically convenient. Preliminary studies indicated that the reaction rate was affected both by oxygen tension and by buffer catalysis but unaffected by ionic strength effects and light. Therefore, the study was designed so that constant oxygen tension was maintained (saturation conditions) and buffers *per se* were not used. Hence, it was assumed that only thermal phenomena and pH contributed to the rate constants obtained.

The reaction followed apparent first-order kinetics with respect to pirbuterol. Figure 2 shows that log concentration *versus* time plots were linear and parallel for each concentration studied (0.5–25 mg/ml) at pH 7. Similar results were also obtained at pH 1, 5, 8.5, and 11. The reaction was then treated as first order in pirbuterol over the entire pH range of 1–14. In Fig. 3, the log concentration of pirbuterol remaining is plotted as a function of time. The slopes of these lines were used to calculate the first-order rate constants (Table I). These log $k$ values can be used to plot a log $k$–pH profile for the reaction (Fig. 4).

The pH of maximum stability was between 1 and 3. Another pH-independent region occurred between pH 10 and 14. The degradation rate

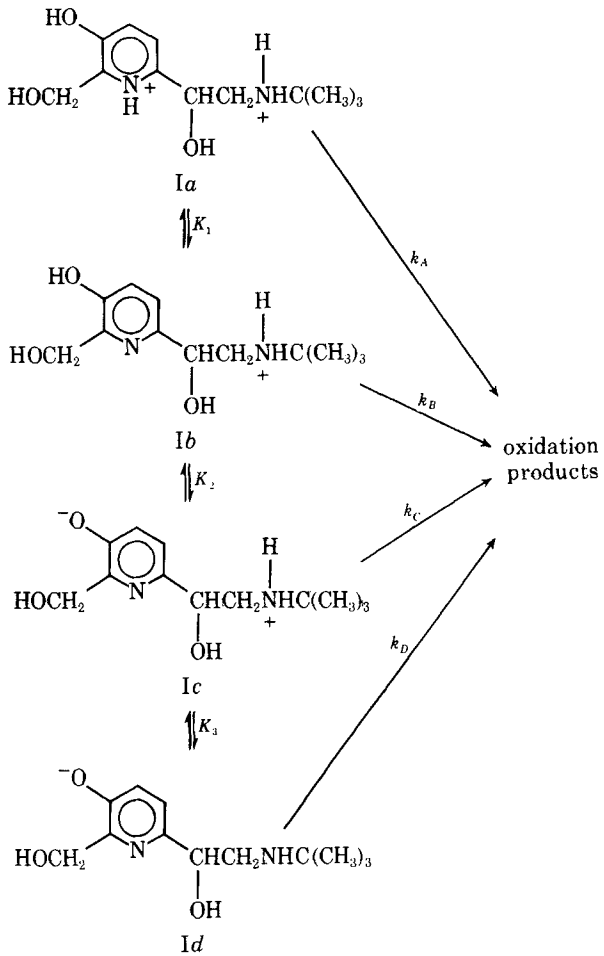
**Table I—Rate Constants for Pirbuterol Degradation Varying as a Function of pH**

| pH | Experimental $k \times 10^4$, $hr^{-1}$ | Experimental log $k + 4$ | Theoretical[a] $k \times 10^4$, $hr^{-1}$ | Theoretical[a] log $k + 4$ |
|---|---|---|---|---|
| 0 | — | — | 1.50 | 0.177 |
| 1 | 1.61 | 0.207 | 1.55 | 0.189 |
| 2 | 1.61 | 0.207 | 1.94 | 0.288 |
| 3 | 4.11 | 0.614 | 4.28 | 0.631 |
| 4 | 7.85 | 0.895 | 7.43 | 0.871 |
| 5 | 8.06 | 0.906 | 8.55 | 0.932 |
| 6 | 13.7 | 1.136 | 12.1 | 1.081 |
| 7 | 33.3 | 1.522 | 31.2 | 1.495 |
| 8 | 53.6 | 1.729 | 53.1 | 1.725 |
| 9 | 49.4 | 1.693 | 48.9 | 1.690 |
| 10 | 36.6 | 1.563 | 36.0 | 1.556 |
| 11 | 33.2 | 1.521 | 32.6 | 1.513 |
| 12 | 32.2 | 1.507 | 32.2 | 1.508 |
| 13 | 32.2 | 1.507 | 32.2 | 1.508 |
| 14 | 32.2 | 1.507 | 32.2 | 1.507 |

[a] Rate constants and equilibrium constants of individual species used in the rate equation for calculation of theoretical curve were: $k_A = 1.50 \times 10^{-4}\ hr^{-1}$, $k_B = 8.24 \times 10^{-4}\ hr^{-1}$, $k_C = 6.00 \times 10^{-3}\ hr^{-1}$, $k_D = 3.22 \times 10^{-3}\ hr^{-1}$, $K_1 = 7.0 \times 10^{-4}$, $K_2 = 8.0 \times 10^{-8}$, and $K_3 = 6.3 \times 10^{-10}$.

**Table II—Ionization Constants of Pirbuterol**

| Functional Group | | 21° | 90° |
|---|---|---|---|
| Pyridine | $pKa_1$ = | 3.0 | 2.8 |
| Pyridol | $pKa_2$ = | 7.0 | 7.1 |
| Secondary amine | $pKa_3$ = | 10.3 | 9.2 |




*Scheme I*

increased with pH between 3 and 6, reaching a pseudomaximum at pH 6, after which the rate rose again until minimum stability was observed at pH 8. This unusual profile can be explained by considering the degradation rates of the various ionic species of pirbuterol whose concentrations change with pH.

Pirbuterol exists as four ionic species. Their pKa's, determined by the titration method described under *Experimental*, are listed in Table II. The equilibria involved in the total reaction are shown in Scheme I.

From these pKa values, it was possible to calculate the degree to which each functional group ionizes as the pH varies (Fig. 5). Figure 6 shows the proportions of ionic species of pirbuterol that are expected to exist in aqueous solution, adjusted to any pH, at 90° [9].

The total rate equation for Scheme I is then:

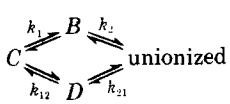
$$k_{obs} = k_A f_A + k_B f_B + k_C f_C + k_D f_D \qquad \text{(Eq. 1)}$$

where:

$$f_A = \frac{[A]}{[A] + [B] + [C] + [D]} \qquad \text{(Eq. 2)}$$

$$f_A = \frac{1}{1 + \frac{[B]}{[A]} + \frac{[C]}{[A]} + \frac{[D]}{[A]}} \qquad \text{(Eq. 3)}$$

[9] Scheme I presents a simplified picture of the ionic equilibria involved. Many more ionic species could theoretically exist (3, 4), the most likely of which would be the totally unionized form. This species would be in equilibrium with species $B$ and $D$ as follows:




where $K_2 = k_1 + k_2$ and $(1/K_3) = (1/k_{12}) + (1/k_{21})$. Based on the relative pKa's of the pyridol and secondary amine, the proportion of unionized species to zwitterion is expected to be very small. Therefore, the mathematical treatment of experimental data neglects the formation of unionized species.

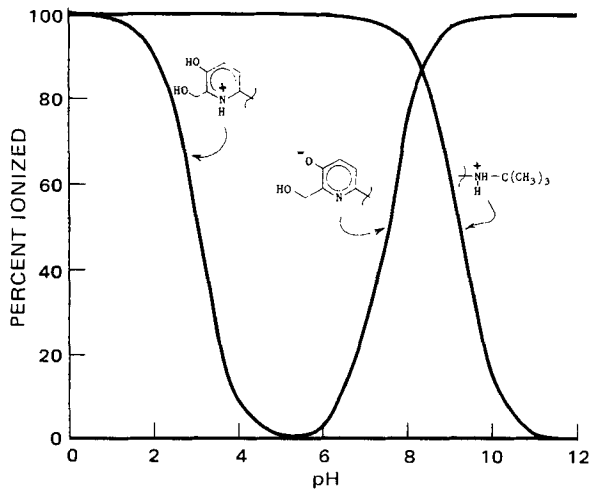



**Figure 5**—*Degree of ionization of pirbuterol at 90° as a function of pH.*

$$f_A = \frac{1}{1 + \frac{K_1}{[H^+]} + \frac{K_1K_2}{[H^+]^2} + \frac{K_1K_2K_3}{[H^+]^3}} \qquad \text{(Eq. 4)}$$

$$f_A = \frac{[H^+]^3}{[H^+]^3 + K_1[H^+]^2 + K_1K_2[H^+] + K_1K_2K_3} \qquad \text{(Eq. 5)}$$

Species $B$, $C$, and $D$ can be treated similarly. Substituting the appropriate ($f_i$) equalities into Eq. 1 yields:

$$k_{obs} = \frac{k_A[H^+]^3 + k_BK_1[H^+]^2 + k_CK_1K_2[H^+] + k_DK_1K_2K_3}{[H^+]^3 + K_1[H^+]^2 + K_1K_2[H^+] + K_1K_2K_3} \qquad \text{(Eq. 6)}$$

In the pH 1–2 region, pirbuterol is diprotonated and $k_A \sim k_{obs}$. Similarly, in the pH 10–12 region, pirbuterol exists as the pyridinolate ion in which case $k_D \sim k_{obs}$. By using these two derived constants, $k_A$ and $k_D$, Eq. 6 can be solved as two simultaneous equations at two pH's with two unknowns, $k_B$ and $k_C$. The latter two rate constants were estimated and optimized in this manner (Table I), and the theoretical pH–rate profile was then plotted and compared with experimental data points (as indicated by the dashed line *versus* observed data points in Fig. 4). The goodness of fit suggests that this model is consistent with experimental data and can be used to explain the unusually shaped pH–rate profile.

The apparent energies of activation were determined at four pH's: 1, 5, 8.5, and 11. As indicated by Fig. 6, essentially one single ionic species exists at any of these pH's; therefore, the $E_a$'s reflect the thermodynamic

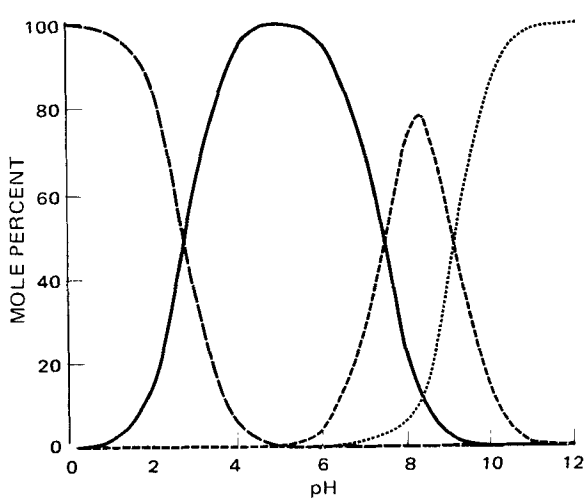
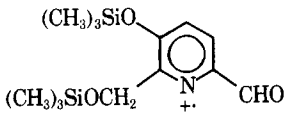



**Figure 6**—*Variation in concentration of pirbuterol species as a function of pH. Key: – –, Ia (diprotonated); —, Ib (monoprotonated); - - - -, Ic (zwitterion); and . . . . , Id (anion).*



III (*m/e* 297)

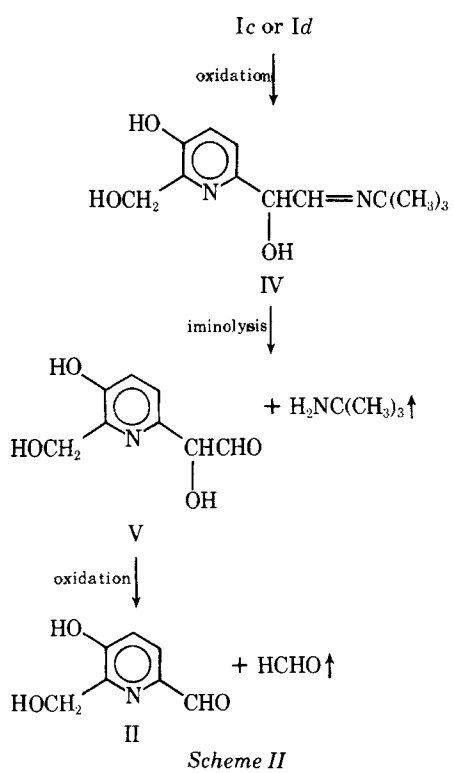
behavior of the respective pirbuterol ion present in that solution. Arrhenius plots of kinetic data collected at 70, 80, and 90° yielded the apparent activation parameters listed in Table III.

As the profile indicates, drug stability is best at pH 2[10], a major factor to be considered in the formulation of liquid products. The relatively small value of the energies of activation indicates a low sensitivity of the reaction rate to temperature. The large negative value of the entropies reflects a significant requirement in molecular orientation for the reaction to occur, presumably imposed by the large bulky *tert*-butyl moiety.

A representative TLC plate of stressed oxidative degradation of pirbuterol is shown in Fig. 7. Five degradation products were observed. The spot migrating to $R_f$ 0.75 (pH 7–12) had a GLC retention time of 2.4 min when derivatized on-column with bis(trimethylsilyl)trifluoroacetamide plus 1% trimethylchlorosilane. The mass spectra[11] of the trimethylsilyl derivative (III) of the degradation product and of II, prepared by unambiguous synthesis, were identical. The prominent peaks in the fragmentation pattern of III (Fig. 8) are listed in Table IV.

Another product resulting from the same reaction sequence is *tert*-butylamine. During the kinetic studies, vapors from the reaction vessels were passed through a saturated aqueous picric acid solution to confirm the presence of the volatile amine. A precipitate was collected in this manner and, upon drying, was analyzed. The melting point, IR spectrum, and elemental analysis corresponded to authentic *tert*-butylamine picrate.

Scheme II is proposed to describe the degradation in solution at pH 7–12 in accordance with the data. The degradation pattern observed at low pH (Fig. 7) indicates that an entirely different mechanism is operative in acidic media. Before a pathway of degradation can be proposed, further identification of degradation products is necessary. Any such mechanism should, however, accommodate the obvious dependence of the reaction rate on the ionization of the ring functionalities.




*Scheme II*

[10] Because $E_a$'s were determined over a small temperature range, extrapolation involves considerable risk. Nevertheless, room temperature data obtained so far do suggest that the $t_{1/2}$ at pH 1–2 is consistent with the predicted value of ~5 years.

[11] LKB-9000 GC–mass spectrometer coupled with a PDP 8 data system.

**Table III—Apparent Thermodynamic Activation Parameters for Pirbuterol Species**

| Ionic Species | Reaction pH | $E_a$, kcal/mole | $\Delta S^{\ddagger}_{298}$, eu |
|---|---|---|---|
| *A* | 1 | 8.8 | −67.5 |
| *B* | 5 | 11 | −58 |
| *C* | 8.5 | 9.5 | −59 |
| *D* | 11 | 11.9 | −53.2 |

**Table IV—Fragmentation Pattern for the Trimethylsilyl Derivative of II**

| *m/e* | Empirical Formula | Comment |
|---|---|---|
| 297 | $C_{13}H_{23}NO_3Si_2^+$ | $M^+$ (molecular ion) |
| 282 | $C_{12}H_{20}NO_3Si_2^+$ | $M^+ - 15$, loss of $CH_3$ from trimethylchlorosilane |
| 253 | $C_{11}H_{19}NO_2Si_2^+$ | $M^+ - 44$, loss of both $CH_3$ and CHO |
| 147 | $C_5H_{15}OSi_2^+$ | $M^+ - 150$, characteristic of trimethylchlorosilane |
| 73, 75 | $C_3H_9Si^+$ | Characteristic of trimethylchlorosilane and its isotope |

Formaldehyde, produced by oxidation of the secondary alcohol (V), can react with the parent amine in a Pictet–Spengler-type reaction to produce the corresponding isoquinoline derivative in the cases of phenylephrine and, to a lesser extent, terbutaline. The parallel reaction, if observed with pirbuterol, would be represented by I (Scheme II) reacting with formaldehyde produced during the formation of II to result in the corresponding pyrido[4,3-*b*]pyridine product. Upon the addition of excess formaldehyde to a solution of pirbuterol (pH ~6), TLC revealed a spot at $R_f$ 0.30, which did not correspond to any observed in Fig. 7. Although this product may have been the cyclized adduct, it was concluded that such a reaction did not occur to any observable extent in the degradation of pirbuterol. This conclusion is likely, since the pyridine nucleus and hydroxyl substitution of pirbuterol should differ significantly in their directing properties from those of the isoquinoline precursors.

Identification of the spots at $R_f$ 0, 0.12, 0.51, and 0.55 has not yet been achieved. Some possible degradation routes lead to *N*-oxide formation, self-condensation, 2,2′-pyridoin compound formation, polymerization of oxidation products, and oxidation of the primary alcohol.

## CONCLUSIONS

The shape of the pH–rate profile (Fig. 4) indicates that each of the four ionic species of pirbuterol oxidizes at a rate independent of the other species present. The diprotonated species appears to be most stable to oxidation, as implied by the profile where optimum stability lies at pH 1–2. In accordance with the pH–rate profile, the rank order of the individual rate constants of the species was observed to be $k_A < k_B \ll k_D$

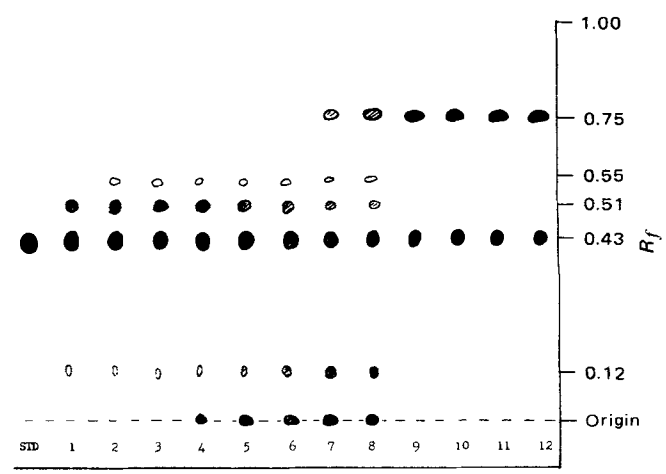



**Figure 7**—*Representative TLC plate showing degradation of pirbuterol at various pH values after* ~$t_{1/2}$.

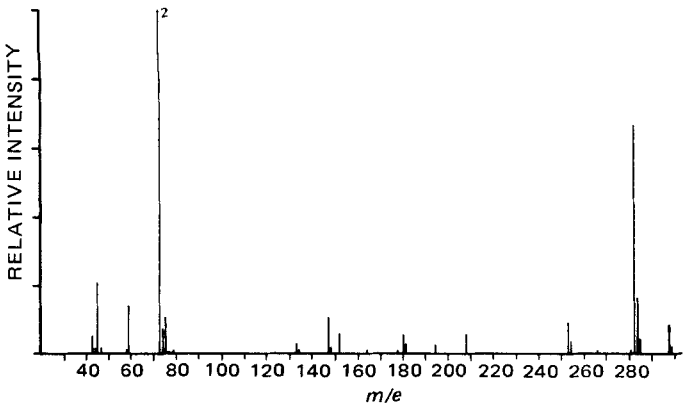



**Figure 8**—*Mass spectrum of the trimethylsilyl derivative of the degradation product from the oxidation of pirbuterol.*

$< k_C$. The experimental values were consistent with the derived rate equation. Arrhenius plots revealed low activation energies (~10 kcal/mole), which explain why conducting the experiments at 90° did not markedly accelerate the oxidation over that predicted (and observed) at room temperature.

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# Prostaglandin Monolayers II: Monomolecular Film Behavior of Dinoprost C-15 Alkyl Esters

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**Abstract** □ Monomolecular film compression–relaxation behavior was examined for select dinoprost C-15 alkyl esters. Higher homologs of the series such as palmitate and decanoate esters yielded stable expanded monolayers that exhibited minimal relaxation of surface pressure during noncompression. Their limiting molecular areas were consistent with a Hirschfelder model projection in which the prostaglandin moiety assumes a horizontal orientation at the interface with its alkyl ester chain oriented vertical to the surface plane. Shorter chain homologs such as hexanoate, valerate, butyrate, propionate, and acetate also formed expanded monolayers but exhibited increased instability with decreased alkyl chain length, as reflected in their lower surface pressure development during compression and significant relaxation of pressure during noncompression. Such instability can be tied to their increased solubility in the subphase solution and higher desorption rate from the interface.

**Keyphrases** □ Dinoprost C-15 alkyl esters, various—monomolecular film compression–relaxation behavior compared □ Films, monomolecular—various dinoprost C-15 alkyl esters, compression–relaxation behavior compared □ Prostaglandins—various dinoprost C-15 alkyl esters, monomolecular film compression–relaxation behavior compared

In recent years, much effort has been directed to elucidating the pharmacological and physiological properties of a new class of biological compounds termed prostaglandins (1–3). Very little attention, however, has been directed toward their possible surface-active properties, even though they have been implicated in various roles portending high surface activity (4–9).

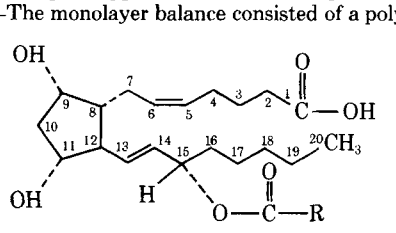
The naturally occurring prostaglandins dinoprost ($F_{2\alpha}$), dinoprostone ($E_2$), $A_1$, and $B_1$ exhibited surface activity in the presence of spread insoluble monomolecular films (10). They in themselves, however, do not tend to form stable monolayers because of their high aqueous solubility. In this article, monolayer film behavior for some long chain alkyl derivatives of dinoprost that do form stable monolayers is reported. This basic and fundamental study should contribute to the limited information available on surface behavior of these ubiquitous and biologically important compounds.

The prostaglandin derivatives selected for monolayer investigation were long chain aliphatic esters of dinoprost attached at the C-15 hydroxyl group (I, R = alkyl). The numbering system generally accepted for prostaglandin-type compounds is with the upper chain carboxylic acid function as the 1-position.

## EXPERIMENTAL

**Materials**—A TLC analysis of the synthesized[1] prostaglandin esters indicated a purity of 99+% for all compounds. Spectroscopic grade hexane–chloroform–methanol (20:2:1) was used as the spreading solvent. Subphase solutions consisted of 0.01 *M* HCl prepared with double-distilled water in an all-glass apparatus with alkaline permanganate.

**Equipment**—The monolayer balance consisted of a polytef[2]-covered

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[1] By Dr. Walter Morozowich, The Upjohn Co.

[2] Teflon, du Pont.