Stability of Prostaglandin E_1 and Dinoprostone (Prostaglandin E_2) under Strongly Acidic and Basic Conditions

R. G. STEHLE ˣ and T. O. OESTERLING *

Abstract □ The stability of prostaglandin E_1 and dinoprostone was investigated at the extremes of the pH range (≤ 3 and ≥ 10) in the sequence prostaglandin E → prostaglandin A → prostaglandin B. The degradation rate is first order with hydrogen-ion and hydroxide-ion concentrations. Separation and analysis of the E prostaglandins were accomplished by TLC and UV spectrophotometry. At the lowest pH values and at elevated or low temperatures, significant amounts of 15-epiprostaglandin E were present. Apparent activation energies for the total dinoprostone loss, calculated from elevated temperature data, were 21 kcal/mole in the strongly acidic region and about 18 kcal/mole at pH 3. Corresponding studies in the alkaline region led to a derived Arrhenius activation energy of 15 kcal/mole with the appearance of significant amounts of 8-iso-prostaglandin E. This difference in activation energies may reflect the different mechanisms operant at high and low pH values.

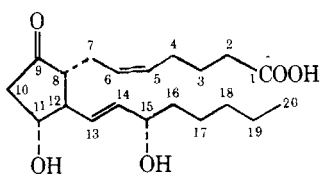
Keyphrases □ Prostaglandins—E_1 and dinoprostone, stability at pH extremes □ Dinoprostone—stability at pH extremes □ Stability—prostaglandin E_1 and dinoprostone at pH extremes □ Degradation—prostaglandin E_1 and dinoprostone at pH extremes □ Oxytocic agents—prostaglandin E_1 and dinoprostone, stability at pH extremes

The biological aspects of prostaglandins dominate the literature on these substances. Prostaglandins appear to be widely involved in the body's biochemistry, with potential applications in such areas as labor induction, contraception, control of ulcers, asthma, and blood pressure regulation. The ready availability from certain forms of *Plexaura homomalla* of A prostaglandin derivatives, which have been converted to E and F prostaglandins (1, 2), has facilitated research on these long chain fatty acids.

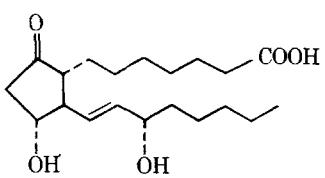
BACKGROUND

The E prostaglandins, those containing the β-hydroxycyclopentanone ring such as dinoprostone[1] (I) ($\lambda_{max} \sim 170$ nm), are important clinically. This β-hydroxy ketone system is unstable and readily undergoes dehydration (3–5) under acidic or basic conditions to A-type prostaglandins, those containing a cyclopentenone ring such as prostaglandin A_2 (III) ($\lambda_{max} \sim 220$ nm). Under basic conditions, the A prostaglandins can isomerize further to B prostaglandins (3) such as prostaglandin B_2 (IV) ($\lambda_{max} \sim 280$ nm).

In addition to this main reaction pathway, the formation of epimers

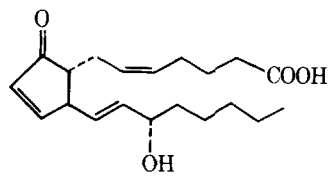


I

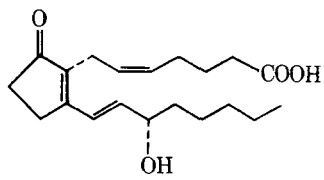


II

[1] Code designation prostaglandin E_2.



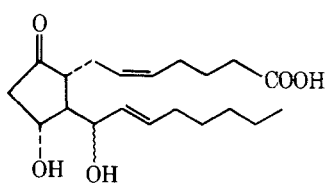
III



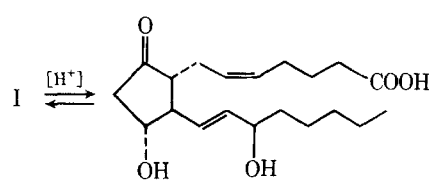
IV

at C-15 may occur under strongly acidic conditions (6) (Scheme I). In mildly to strongly basic media, the E prostaglandin can form an equilibrium mixture with its C-8 isomer (7) (Scheme II). The 13,15-allylic rearrangement product of A prostaglandins was found in acidic reaction mixtures (8), and it was implied that E prostaglandins can also undergo 13,15-rearrangement under similar conditions to small amounts of, for example, V. Other limited evidence (8) indicates that the 13,15-prostaglandin A rearrangement product also can dehydrate to give "minor elimination products."

A kinetic study of the prostaglandin E → prostaglandin A → prostaglandin B reaction as a function of pH and temperature was reported recently (9), in which the total amounts of E, A, and B prostaglandins present were followed by UV spectrophotometry. This procedure did not, however, account for the concentrations of epimers and other prostaglandin derivatives with the same or similar absorption properties. At low pH values (<~2.5), the dehydration rate was directly proportional

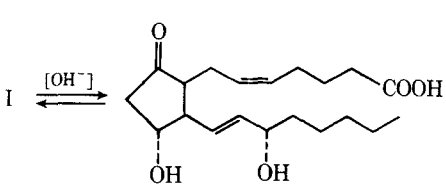


V



VIII

Scheme I



VII

Scheme II

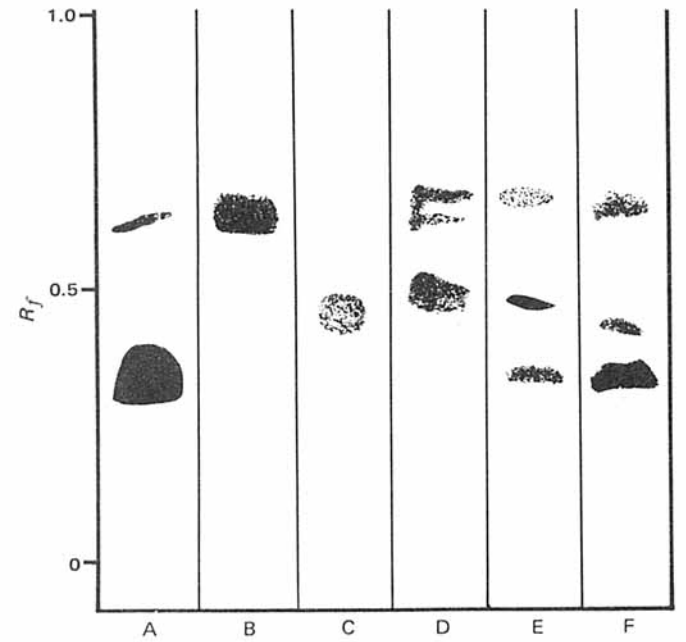


Figure 1—*Thin-layer chromatograms (on silica gel) of prostaglandins developed in ethyl acetate–ethanol–acetic acid (100:1:1). The heavily spotted I sample shows a small amount of III. Isomers found in acidic and basic degradation mixtures are clearly different. The acidic solution was a 12-hr, 37°, pH 1.1 sample; the basic solution was from 0.01 N KOH, approximately 10 min at 37°. Prostaglandins of the 1 and 2 series are not resolved in this TLC system. Key: A, I; B, III; C, VII; D, VIII (impure); E, acidic solution; and F, basic solution.*

to the hydrogen-ion concentration. The calculated activation energy, E_a, values for the dehydration process were 18 kcal/mole at low pH values and 24 kcal/mole at intermediate (neutral) pH values. The E_a for the prostaglandin A → prostaglandin B process was 20 kcal/mole, and there was a difference in the dehydration rate constants for prostaglandin E_1 (II) and I in the pH 3–10 range.

In another study (10), a total rate equation for I decomposition was developed. The high temperature data were consistent with literature data (9). The room temperature studies, however, led to a more thorough interpretation of the kinetic data in the pH 4–9 range.

In previous work (11), the prostaglandin E → prostaglandin A → prostaglandin B reaction was studied in dilute sodium hydroxide solutions. Isomerization (prostaglandin A → prostaglandin B) constants were measured by: (*a*) monitoring the development of the B prostaglandin peak at 283 nm when starting with alkaline solutions of the corresponding A prostaglandin, and (*b*) reacting E prostaglandins under conditions identical to those used for the A prostaglandins and again measuring the appearance of the prostaglandin B peak at 283 nm. Estimation of dehydration (prostaglandin E → prostaglandin A) and isomerization (prostaglandin A → prostaglandin B) rate constants involved the fitting of product–time data to the rate equations with an iterative nonlinear least-squares computer program.

In those studies (11), the apparent dehydration rate constants were approximately six times the values of the corresponding prostaglandin A → prostaglandin B rate constants. For prostaglandin A_1 → prostaglandin B_1 and prostaglandin A_2 → prostaglandin B_2, the respective Arrhenius activation energies were 15.5 ± 1.2 and 13.4 ± 0.8 kcal/mole.

This report focuses mainly on the kinetics of the total degradation of I and II as a function of temperature and pH and attempts to evaluate the relative contribution of the various isomerization reactions.

EXPERIMENTAL

White crystalline II[2], mp 113–115°, and I[2], mp 65–68°, were used. The estimated purity in each case was ≥98% by TLC. White, crystalline 8-isoprostaglandin E_1[2] (VI), mp 73–75°, and 8-isoprostaglandin E_2[2] (VII), mp 107–108°, showed one spot by TLC.

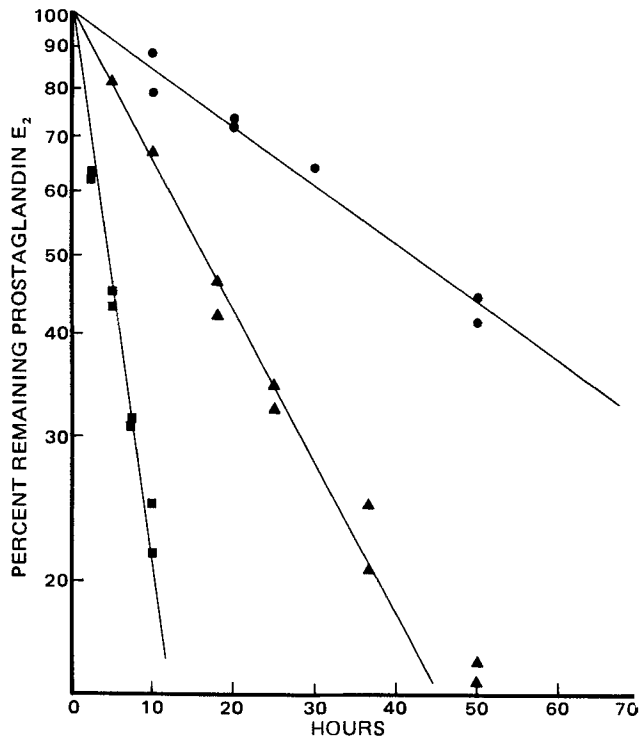


Figure 2—*Kinetic data for total disappearance of I from aqueous solution, pH 2.05. Key:* ●, *47°;* ▲, *57°; and* ■, *70°.*

Low pH Studies—The total loss of I from solutions of low pH was measured by assaying the remaining I as a function of time. A 0.25-ml volume of ethanolic I, 50 mg/ml, was slowly added, with vigorous agitation, to 25 ml of the aqueous solutions (0.1 *N* HCl, pH 1.10; 0.01 *N* HCl, pH 2.05; and 0.1 *M* citrate buffer, pH 2.99) at the reaction temperatures. The resulting 0.5-mg/ml solutions were well within the room temperature solubility of I in solutions below pH 4 (12).

Samples of 2 ml were withdrawn from the 0.5-mg/ml I solution at zero time and at appropriate time intervals. Each sample was extracted twice with water-saturated ethyl acetate; the organic phase was removed after vigorous shaking and centrifugation. The cumulative extract was evaporated to dryness under nitrogen and vacuum, and ethanol was added to make 100 μl. Samples of 10 μl of the concentrate were analyzed by TLC.

High pH Studies—Studies at pH > 10 were carried out in various concentrations of sodium or potassium hydroxide. The system was adjusted to an ionic strength of 0.4 with potassium chloride. Runs were performed at two initial prostaglandin concentrations to verify that the overall loss followed first-order kinetics.

Solutions of 0.1 mg of I or II/ml were prepared in 25-ml stoppered erlenmeyer flasks with aqueous sodium hydroxide (0.002–0.010 *N*) containing sufficient potassium chloride to adjust the ionic strength to 0.4. Acidified 10-ml aliquots, removed at various times, were quantitatively extracted with three 10-ml portions of methylene chloride or water-saturated ethyl acetate. The cumulative organic phase was dried under vacuum with a nitrogen stream, concentrated, and treated as described under TLC.

Rate constants for the total disappearance of I from alkaline solutions between pH 10.3 and 11.5 were obtained by dissolving I at a concentration of 0.5 mg/ml (1.42×10^{-3} *M*) in 0.0025–0.010 *N* KOH and adjusting to ionic strength 0.4 with potassium chloride. The pH of the solution was then read to within ±0.02 unit[3] at the reaction temperature since the II concentration was not negligible compared to the hydroxide-ion concentration. Samples of 2 ml were withdrawn at suitable times after the initial mixing, acidified to pH 3 with dilute hydrochloric acid, and prepared for quantitative TLC as already described. Three 3-ml volumes of ethyl acetate were sufficient for quantitative extraction. The pH was read again after the final sample was withdrawn.

TLC—Andersen (13) evaluated the ability of many solvent systems to separate A and B prostaglandins and epimers of E prostaglandins from

[2] Supplied by research laboratories at The Upjohn Co.

[3] Fisher Accumet, model 310, Fisher Scientific Co., Pittsburgh, PA 15219.

Table I—Rate Constants for Total Loss of I from Acidic Media

pH	Temperature	K, $sec^{-1} \times 10^6$	E_a, kcal/mole
1.10	17°	1.51, 1.50	20.7 ± 0.1
	37°	13.9, 14.6	
	47°	41.9, 42.2, 44.7	
	56°	94.7, 96.9	
	70°	344, 351	
2.05	37°	1.73, 1.75	21.2 ± 0.2
	47°	4.92, 4.28	
	57°	12.5, 11.9	
	70°	42.8, 42.5	
2.99	37°	1.25	18.4 ± 0.3
	49°	3.64	
	57°	7.19	
	70°	22.1	

the natural E prostaglandins. It was decided that quantitative TLC would provide a reliable and accurate method of analysis and separation of I or II from all degradation products. Alcoholic concentrates of reaction mixtures were spotted onto prescored silica gel plates[4], which were then developed in ethyl acetate–acetic acid–ethanol (100:1:1) (13). This system provided adequate separation of E prostaglandins and their isomers, particularly 15-epiprostaglandin E_2 (VIII) and VII (Fig. 1).

Since high laboratory humidity (>50%) resulted in a significant loss in resolution, the plates were activated by heating overnight at 70° and stored and spotted in a constant (15–20%) humidity room. After development, one end of the prescored plate, reserved as a marker to locate the E prostaglandin in the developed kinetic samples, was broken off, sprayed with 15% ammonium sulfate, and charred on a hot plate. Once located, an unknown sample was scraped into a sintered- glass funnel and eluted with methanol into a 10-ml volumetric flask. A "plate blank" served to subtract out any background absorbance due to plate binders and/or colloidal silica gel particles.

Each flask was treated with 1 ml of 5 *N* KOH, virtually instantaneously converting the E prostaglandin to the corresponding B prostaglandin, and diluted to volume with methanol. After 10 min, the samples were scanned from 350 to 250 nm against a potassium hydroxide–methanol blank on a recording spectrophotometer[5]; results were compared to a standard curve (λ_{max} 278 nm) for E prostaglandin converted to B prostaglandin in aqueous methanolic potassium hydroxide.

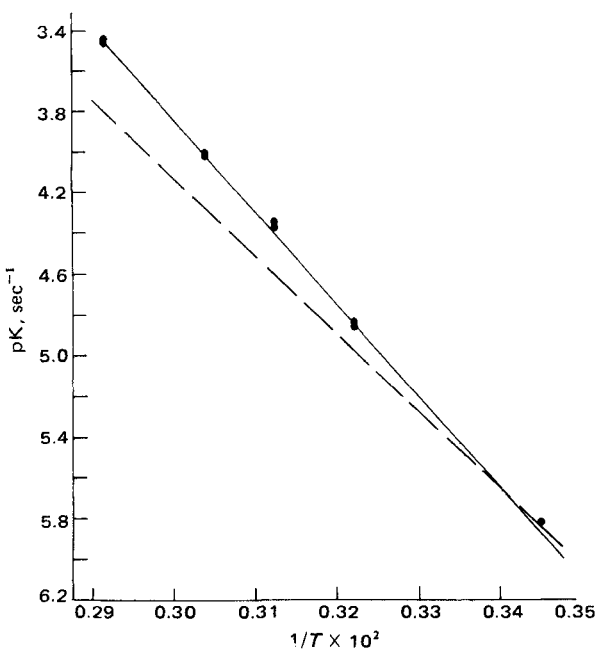


Figure 3—*Arrhenius plot of I degradation at pH 1.10. Data of Monkhouse* et al. *(9), pH 1.2, are included for comparison (dashed line); K refers to overall apparent first-order rate constant.*

[4] Silica gel GF, 250 μm, Analtech, Newark, DE 19711.
[5] Zeiss DMR 21, Carl Zeiss, Oberkochen, West Germany.

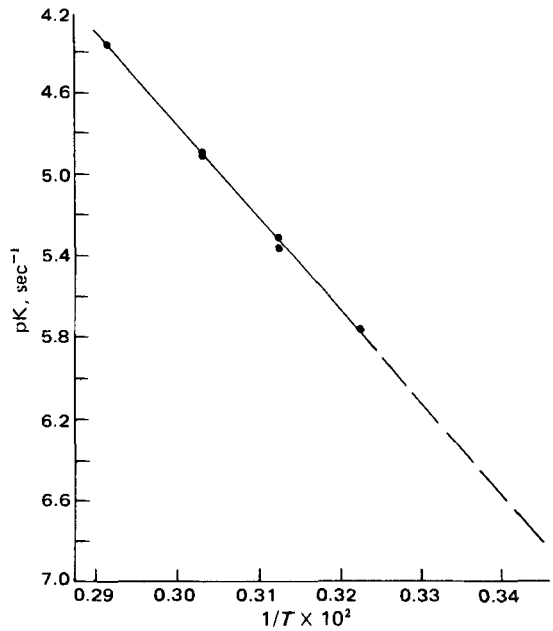


Figure 4—*Arrhenius plot of I degradation at pH 2.05.*

RESULTS

Figure 2 shows typical first-order time data for the total disappearance of I from solution at pH 2.05. Data were plotted as percent of the initial assay. No significant change in pH occurred during a run in acid media. Table I lists the low pH degradation data for I. These data are also summarized in Figs. 3–5.

The pH 1.2 dehydration rate data of Monkhouse *et al.* (9) were included for comparison in Fig. 3. The present E_a values at the lower pH values, where appreciable epimerization and possible rearrangement products were observed, are essentially identical, and higher than the 18-kcal/mole value previously found (9). The E_a observed at pH 2.99, however, where negligible epimerization, *etc.*, was seen, agreed with that reported (9) for dehydration alone.

The rate of total loss of I was proportional to the hydrogen-ion concentration in the pH 1–2 region but not around pH 3. Above about pH 2.5, specific hydrogen-ion catalysis decreases in relative importance, a significant part of the total loss of E prostaglandin being due to water or buffer catalysis. Under the most severe conditions encountered in the oral administration of an E prostaglandin (37°, pH 1–1.5), roughly 5% of the prostaglandin would be lost per hour from nonenzymatic causes.

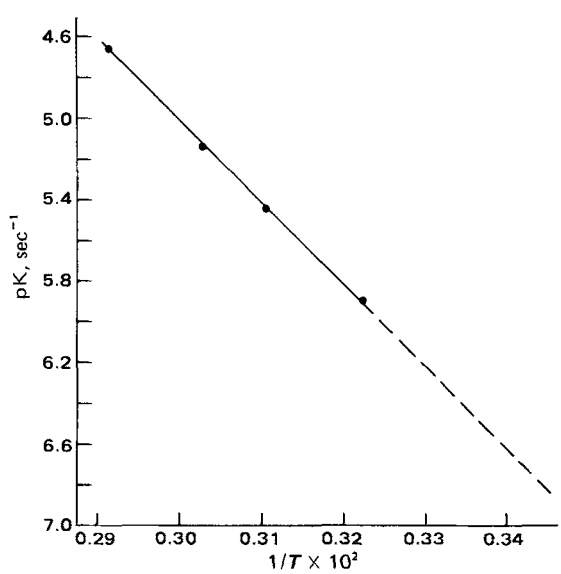


Figure 5—*Arrhenius plot of I degradation at pH 2.99.*

Table II—Effect of pH and Temperature on Apparent First-Order Rate Constants of Alkaline Dehydration of I and II and Their 8-Isomers at 0.1 mg/ml

Prostaglandin	Temperature	Sodium Hydroxide Concentration, *N*	pOH	*K*, sec^{-1} × 10^4
II	37°	0.002	2.70	4.40
II	37°	0.004	2.40	7.21
II	37°	0.006	2.22	10.0
II	37°	0.008	2.10	17.3
II	37°	0.010	2.00	19.3
VI	37°	0.004	2.40	8.07
I	37°	0.002	2.70	4.09
I	37°	0.004	2.40	9.30
I	37°	0.006	2.22	11.8
I	37°	0.008	2.10	16.9
I	37°	0.010	2.00	18.2
II	27°	0.004	2.40	3.36
II	47°	0.004	2.40	15.4
II	57°	0.004	2.40	35.2
VII	37°	0.004	2.40	8.26
I	27°	0.004	2.40	3.17
I	47°	0.004	2.40	16.6
I	57°	0.004	2.40	29.1

Table II lists first-order data for the loss of I and II from alkaline solutions made with sodium hydroxide. There is little difference between the rate constants measured for an 8-iso-E prostaglandin and the E prostaglandin itself. Moreover, no significant difference is seen between I and II. This result is consistent with the previous data (9) where the I and II curves appeared to be converging at pH 10.

Figure 6 shows the rate dependence of the overall loss of E prostaglandin on temperature in 0.004 *N* NaOH. Arrhenius activation energies from these data are 15.4 ± 0.5 kcal/mole for II and 14.3 ± 1.5 kcal/mole for I where the ± figure is the least-squares standard deviation. The lower E_a values at alkaline pH's may reflect the different mechanisms operant at low and high pH values. At high pH, the mechanism is probably the abstraction of an activated hydrogen alpha to the carbonyl group or from the enol form of the prostaglandin; in strongly acid solutions, attack of a suitable site, such as C-11, by a proton forming a good leaving group (HOH) can be the first step in the elimination process.

Table III presents results obtained in I stability studies in potassium hydroxide solution at 0.5 mg/ml. Figure 7 summarizes the 37° data contained in Tables II and III; net hydroxide-ion concentrations were estimated as initial hydroxide-ion concentrations for the 0.1-mg/ml prostaglandin solutions, and as $[OH^-] = [OH^-]_0 - [I]_0 = [OH^-]_0 - 1.42 \times 10^{-3}$ in the 0.5-mg/ml runs in potassium hydroxide, where the subscript indicates initial values.

The least-squares slope is 0.95 for the II data and 0.94 for I when sodium hydroxide is the alkaline medium. The corresponding slope for the I solutions in potassium hydroxide is 1.17. When more accurately plotted against the average pH during the run (Fig. 8), the dependence of the rate constant in potassium hydroxide becomes 0.93. Therefore, the overall loss rate constant is directly proportional to the hydroxide-ion concentration in the pH 10.3–11.5 range. The data in Fig. 7 suggest that the process is first order with respect to the prostaglandin E concentration in the 0.1–0.5-mg/ml range.

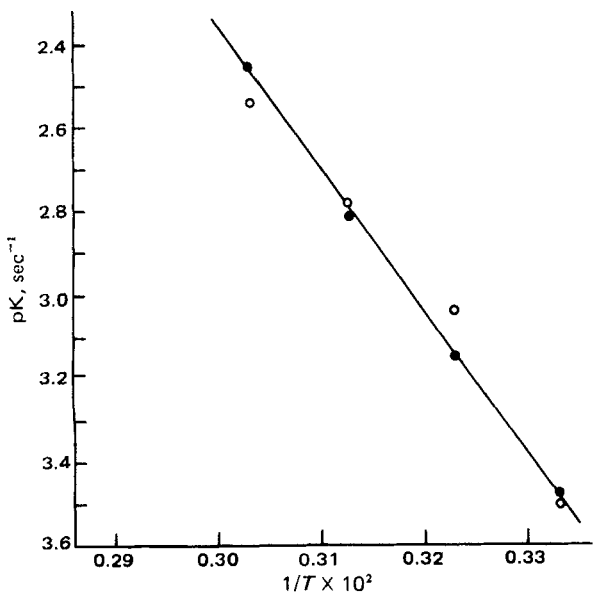


Figure 6—*Effect of temperature on II (●) and I (○) degradation in sodium hydroxide solutions.*

Table III—Rate Data for I in Alkaline Media (Potassium Hydroxide) at 37°; $[I]_0 = 1.42 \times 10^{-3}$ *M*

Potassium Hydroxide Concentration, *N*	Initial pH	Final pH	Average pH	*K* sec^{-1} × 10^4	pK
0.0025	10.47	10.23	10.35	1.85	3.73
0.0025	10.47	10.23	10.35	1.81	3.74
0.0030	10.66	10.37	10.52	3.25	3.49
0.0035	10.90	10.63	10.77	3.98	3.40
0.0035	10.90	10.63	10.77	3.94	3.40
0.0040	10.90	10.70	10.80	5.43	3.27
	10.90	10.70	10.80	5.28	3.28
0.0050	11.19	10.92	11.06	9.31	3.03
	11.19	10.92	11.06	9.07	3.04
0.0060	11.31	11.04	11.18	11.2	2.95
	11.31	11.04	11.18	10.9	2.96
0.0075	11.46	11.22	11.34	18.0	2.75
	11.46	11.22	11.34	17.6	2.75
0.010	11.54	11.42	11.48	19.6	2.71
	11.54	11.42	11.48	19.6	2.72

DISCUSSION

Thin-layer chromatograms of extracts from acidic reaction mixtures show spots other than those attributable to prostaglandins E, A, and B and 15-epiprostaglandin E. This result is particularly true of the most acidic mixtures (pH 1.10) at the higher reaction temperatures (60 and 70°). One spot, which appeared just above the 15-epiprostaglandin E on the TLC plate, probably represents the 13,15-rearrangement product; it never appeared to be more than a few percent of the total material present.

Scans of these solutions in the UV range showed a peak at 283 nm, implicating formation of B prostaglandins in acidic media. Occasionally, two spots appeared above the A/B prostaglandin spot. One apparently was the 15-epiprostaglandin A/B spot; the other was a degradation product with a λ_{max} of ~325 nm and was observed in various kinetic studies[6] (9). This spot may be the 13,15-dehydration product of prostaglandin A containing a highly conjugated trienone system (IX).

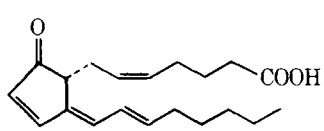
Schematic Model for Reaction Products—With these considerations in mind, a model illustrating the various pathways involved in the total loss of E prostaglandins from aqueous solution may be constructed to aid in the interpretation of experimental data. Although this paper deals with the total loss of the E prostaglandin by whatever paths exist, analogous routes for losses of A and B prostaglandins from acidic solutions may also be proposed as in Scheme III. Scheme IV shows the similar possibilities in strongly basic media.

For the prostaglandin E (E), 15-epiprostaglandin E (15E), and 13,15-rearrangement product (E*) compartments (in the case of acid solutions), which are assumed isolated from the remaining scheme by irreversible reactions, the differential equations for the system, with the assumption that all processes are first order, are:

$$\frac{d[E]}{dt} = (-k_1 - k_5 - k_8)[E] + k_{-5}[15E] \quad \text{(Eq. 1)}$$

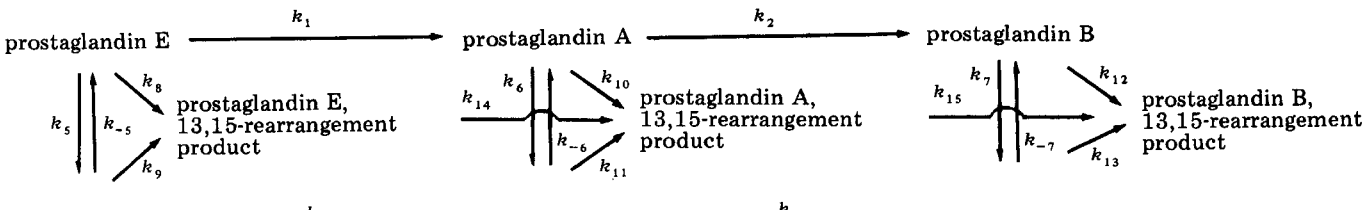
$$\frac{d[15E]}{dt} = k_5[E] + (-k_3 - k_{-5} - k_9)[15E] \quad \text{(Eq. 2)}$$

$$\frac{d[E^*]}{dt} = k_8[E] + k_9[15E] - k_{14}[E^*] \quad \text{(Eq. 3)}$$

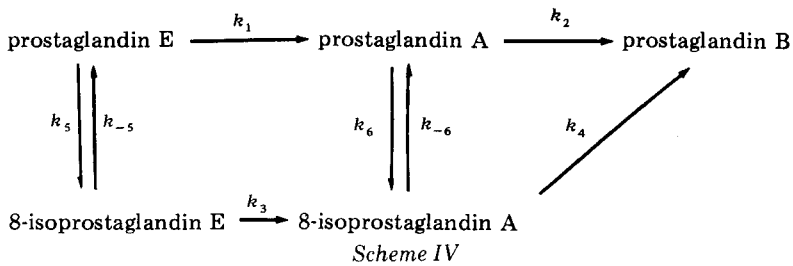


IX

[6] T. O. Oesterling, unpublished observations.



Scheme III



Scheme IV

All k's are, in reality, pseudo-first-order rate constants, containing factors accounting for specific hydrogen-ion, water, and buffer catalyses, and remain constant in any given experiment. Because of the assumption of irreversibility (k_8 and k_9), Eqs. 1 and 2 may be solved as a separate system by using the Laplace transform method, as recently applied to pharmacokinetic systems (14). When assuming that the starting material is pure prostaglandin E at concentration E_0, the total solution is:

$$E(t) = E_0\left[\frac{(k_3 + k_{-5} + k_9 - \alpha)e^{-\alpha t}}{\beta - \alpha} + \frac{(k_3 + k_{-5} + k_9 - \beta)e^{-\beta t}}{\alpha - \beta}\right] \quad \text{(Eq. 4)}$$

$$15E(t) = \frac{k_5 E_0}{\alpha - \beta}(-e^{-\alpha t} + e^{-\beta t}) \quad \text{(Eq. 5)}$$

where:

$$\alpha = \frac{(k_1 + k_3 + k_5 + k_8 + k_{-5} + k_9) + \sqrt{(k_1 + k_3 + k_5 + k_8 + k_{-5} + k_9)^2 - 4(k_1k_{-5} + k_1k_9 + k_5k_9 + k_8k_{-5} + k_8k_9 + k_1k_3 + k_3k_5 + k_3k_8)}}{2} \quad \text{(Eq. 6)}$$

$$\beta = \frac{(k_1 + k_3 + k_5 + k_8 + k_{-5} + k_9) - \sqrt{(k_1 + k_3 + k_5 + k_8 + k_{-5} + k_9)^2 - 4(k_1k_{-5} + k_1k_9 + k_5k_9 + k_8k_{-5} + k_8k_9 + k_1k_3 + k_3k_5 + k_3k_8)}}{2} \quad \text{(Eq. 7)}$$

In all cases, the data for the total disappearance of E from solution suggested that:

$$E(t) = E_0e^{-Kt} \quad \text{(Eq. 8)}$$

where K is some composite of the individual rate constants. If this assumption is made, solution of Eq. 2 gives the biexponential expression:

$$15E(t) = \frac{k_5E_0}{-K + k_{-5} + k_9 + k_3}[e^{-Kt} - e^{-(k_{-5}+k_9+k_3)t}] \quad \text{(Eq. 9)}$$

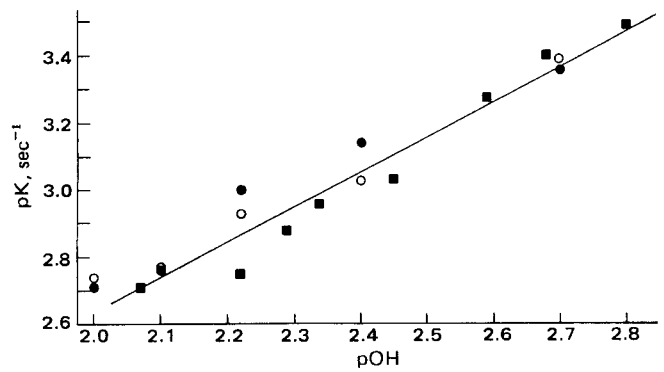


Figure 7—*Effect of OH^- concentration on degradation of II in sodium hydroxide (●), I in sodium hydroxide (○), both 0.1 mg/ml, and I in potassium hydroxide solution (■), 0.5 mg/ml.*

Comparison with Eq. 5 reveals that $\alpha - \beta = -K + k_{-5} + k_9 + k_3$, $\beta = K$, and $\alpha = k_{-5} + k_9 + k_3$. Therefore, the expression for $E(t)$ becomes:

$$E(t) = E_0e^{-\beta t} \quad \text{(Eq. 10)}$$

The apparent first-order total disappearance of I or II is, therefore, a composite of all individual rate constants in the system. The overall rate constant will, depending on the amount of epimerization, *etc.*, be greater than k_1, the dehydration rate constant. This result explains why the E_a values at pH 1–2 were greater than those seen previously (9) in the same pH region. Furthermore, in a 4° run with I in 0.1 *N* HCl (not included in Fig. 3 or Table I), a significant portion of the total loss was in the form of the 15-epimer. The epimerization rate was, therefore, more comparable to the dehydration rate to III. This could show up as a changed slope on the log K *versus* $1/T$ plot, particularly if the activation energy for epimerization is significantly less than that for dehydration. In the extreme case, where the dehydration is negligible compared to epimerization, the E prostaglandin would form an equilibrium mixture with its 15-epimer. At pH 3, however, the amount of epimer and 13,15-rearrangement product appeared to be negligible, *e.g.*, k_5 and k_8 approached zero. Equation 10 then becomes:

$$\lim_{k_5,k_8 \to 0} E(t) = E_0e^{-t/2}[(k_1 + k_3 + k_{-5} + k_9) - \sqrt{(k_1 + k_3 + k_{-5} + k_9)^2 - 4(k_1k_{-5} + k_1k_9 + k_1k_3)}] \quad \text{(Eq. 11a)}$$

$$E(t) = E_0e^{-k_1t} \quad \text{(Eq. 11b)}$$

The total loss of the E prostaglandin should, therefore, be dependent only on k_1, the dehydration rate constant around pH 3. Similar predictions would apply to strongly alkaline reaction mixtures, with 8-isoprostaglandin E replacing 15-epiprostaglandin E and with k_8–$k_{15} = 0$. In the present experiments, no more than 5–10% 8-isoprostaglandin E was formed under any conditions (Fig. 1). The reaction environment necessary to produce enough isomer to show a significant discrepancy between k_{obs} and ($k_{dehydration} = k_1$) would produce an overall rate too rapid to be measured accurately by the present experimental technique.

Comparisons with thin-layer chromatograms of standard methyl and ethyl esters of E prostaglandins showed that negligible esterification occurs in the presence of the small amount of alcohol vehicle in which the free acid prostaglandin is introduced to the aqueous buffer system. The methyl or ethyl ester of an E prostaglandin has a slightly greater R_f than the respective 15-epimer.

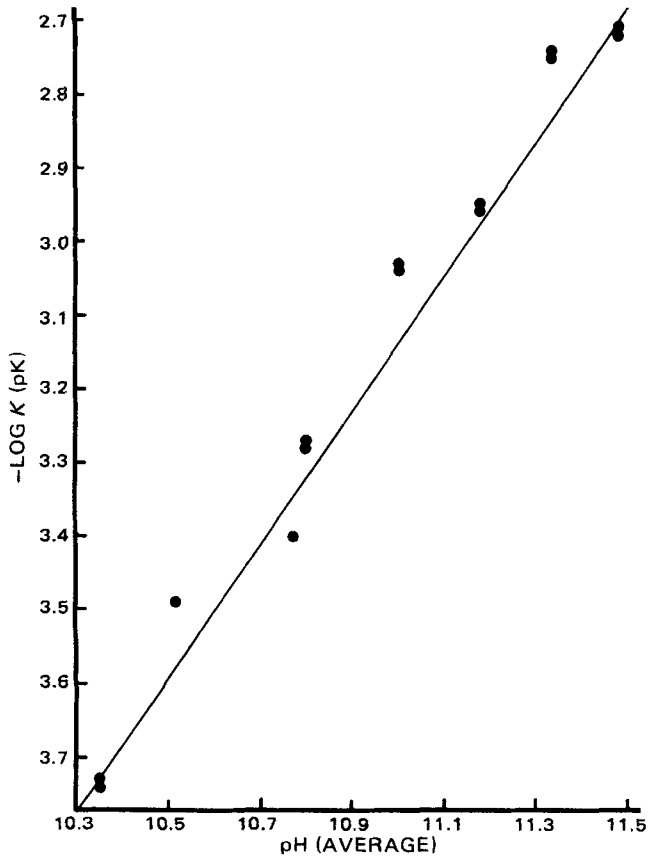


Figure 8—*Degradation rate of I in potassium hydroxide solutions as a function of the average pH during the individual kinetic run (0.5 mg/ml).*

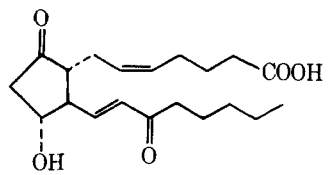
Proposed Experimental Testing of Model—A primary requisite for testing the model depicted in Schemes III and IV is the development of a rapid, sensitive assay to measure the amounts of the various minor degradation products as a function of time. Recent work in high-performance liquid chromatography (HPLC) of prostaglandins (15, 16) closely related in structure revealed a promising technique for analysis of minor prostaglandin components in solution, provided appropriate standards are available. Given a workable HPLC assay, key experiments with E prostaglandins may be run to measure simultaneously the individual reaction products in the scheme on both relative and absolute bases.

Furthermore, if one can assume independence of the various reactions (*e.g.*, 15-epimerization and dehydration), the degradation of prostaglandins, in which one or more of these reactions is blocked through lack of a necessary group, may be studied. For example, the 15-oxo derivative of I (X), a primary metabolite (17–19), could not epimerize at C-15 or rearrange at C-13–C-15; therefore, pure dehydration rate constants could be obtained. Prostaglandin $F_{1\alpha}$ or dinoprost[7] (XI) has hydroxyl groups at C-9 and C-11, obviating consideration of the dehydration rate which is so rapid in E prostaglandins as to hamper measurement of the other slower, but probably more comparable, reaction rates.

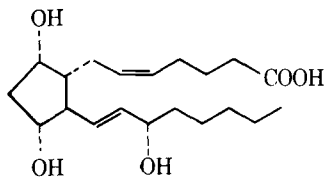
Of particular interest is the epimerization equilibrium described by the relative values of k_5 and k_{-5}, which become appreciable only at very low pH values. Studies with XI and its 15-epimer could, therefore, lead to reasonable estimates of values of k_5, k_{-5}, and, possibly, k_8 and k_9. Values of all derived rate constants could then be inserted appropriately in Eq. 10 for the E prostaglandin, and the calculated value of K, the overall rate constant, could be compared to the observed value.

If the model is found to be seriously in error, changes in Scheme III would be necessary. For example, if formation of the 13,15-rearrangement product proved to be an equilibrium process, additional rate constants would be required. The modified forms of Eqs. 1–3 would then be resolved as a unit.

One criticism of the present model is that it does not assign formation



X



XI

mechanisms for the various products, as did Thompson *et al.* (10). The problem is the consideration of more than one reaction and product. The rationale for avoiding the detailed consideration of reaction mechanisms here is that specific acid (H^+) and base (OH^-) catalyses are probably responsible for virtually all reactions observed at these extremes of pH. In acidic solutions, the relative contributions of the different possible mechanisms change appreciably only when the pH rises to ~3, leading to a different product balance and apparent energy of activation. The same situation holds true for low temperature studies (*e.g.*, 4° at pH 1.1).

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[7] Code designation prostaglandin $F_{2\alpha}$.