Kinetics of Dehydration and Isomerization of Prostaglandins E_1 and E_2

D. C. MONKHOUSE[▲], L. VAN CAMPEN, and A. J. AGUIAR

Abstract □ The degradation of prostaglandins E_1 and E_2 was studied at 60° at a pH range of 1–10. A consecutive first-order reaction appeared to be operative above pH 4 for the dehydration and rearrangement reactions. Below pH 3 the dehydration appeared to be first order with respect to hydrogen-ion concentration. The rate of dehydration of E_2 was two to three times faster than that of E_1 under the same conditions over a pH range of 4–10. The rearrangement of E_1 and E_2 proceeded at approximately the same rate at pH 10. The rate of E_2 rearrangement progressively exceeded that of E_1 with a decrease in pH such that at pH 6, E_2 rearranged at a rate 15 times that of E_1. These data suggested that the cis-Δ^5 double bond participated actively in the rearrangement reaction of E_2, and was responsible for the greater reactivity of this molecule compared to E_1.

Keyphrases □ Prostaglandins E_1 and E_2—dehydration and isomerization kinetics, pH effect □ Dehydration and rearrangement kinetics—prostaglandins E_1 and E_2 □ Kinetics, dehydration and rearrangement—prostaglandins E_1 and E_2 □ UV spectrophotometry—monitoring, dehydration and isomerization of prostaglandins E_1 and E_2 □ pH effect—dehydration and isomerization kinetics of prostaglandins E_1 and E_2

Prostaglandins affect a wide range of physiological processes, from the contraction of the uterus to secretion from the stomach wall. This diversity of action accounts for the great current interest in their use as pharmacological agents.

The application of prostaglandins in a number of areas has been severely hampered by their apparent instability in solution. Karim *et al.* (1) measured the biological activity of saline solutions of prostaglandins stored at various pH values at room temperature. The PGE compounds showed 25–40% loss of biological activity after 60 days at pH 5–7. Andersen (2) reported 5–20% conversion to PGA_1, measured spectrophotometrically, in methanolic solutions of PGE_1 after 1 month at 5–10°. Brummer (3) found that, for E_2 in ethanolic solution stored for 4 weeks at 4°, there was no detectable loss of total $E_2 + A_2$; after 6 months, 33% of the E_2 had been converted to PGA_2 and bioassay of the solution showed only 50% potency. As a result of this study, it was recommended that PGE solutions prepared for clinical use be stored as concentrated alcoholic solutions in ampuls. These can be stored for long periods at −20° and diluted with sterile isotonic saline as required within 24 hr. of use.
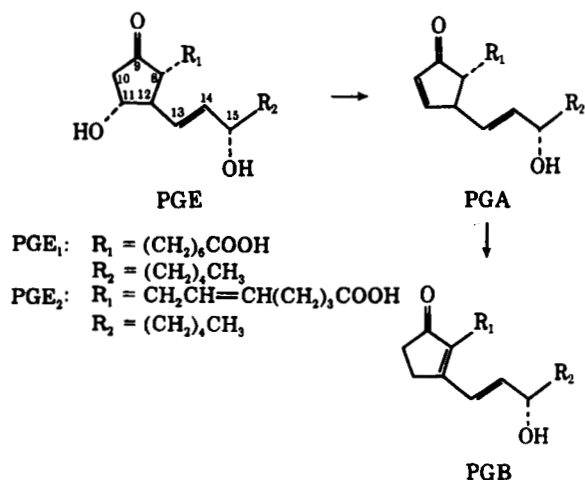
The β-hydroxyketone moiety of the PGE compounds[1] is extremely reactive. Loss of protons at C-8 or C-10 occurs very readily in both acid and alkaline media. The loss of a proton at C-10 may result in a negative charge, expulsion of the hydroxyl group at C-11, and

formation of a Δ^{10} double bond (PGA compound). Under base treatment the PGA compounds undergo internal rearrangement of the double bond ($\Delta^{8,12}$) to form the relatively stable PGB compounds containing a dienone chromophore (Scheme I).

Treatment of PGE_1 (1 mg./ml. in methanol) with 1 ml. of 1 N HCl resulted in 80% conversion to a mixture of PGA_1 and methyl PGA_1 after standing for 16 hr. in the dark at room temperature. Systematic studies (4) of the stability of methanolic solutions of PGE_1 in various media for 6 hr. at room temperature revealed that PGA_1 formation was only evident at low pH values (<3) or if the buffer exceeded pH 7.4. Thus, in buffers of carbonate–borate–potassium hydroxide (pH 10.0) or sodium acetate–potassium hydroxide (pH 11–11.5), approximately 50–60% conversion of PGE_1 to PGA_1 was obtained. At the higher pH value, 20% PGE_1 passed into the PGB form after 6 hr. at room temperature. In the presence of oxalic acid (pH 2.5), saturated ammonium chloride in biphthalate buffer (pH 4), potassium acetate–acetic acid (pH 5), or sodium acetate (pH 7.4), PGE_1 was found to be stable (<10% conversion to PGA_1) for 6 hr. at room temperature; longer exposure, however, did result in significant PGA formation.

Oesterling (5) measured the rate of dehydration of E_1 and E_2 and the rate of isomerization of A_1 and A_2 in dilute sodium hydroxide solutions. These processes exhibited a first-order dependency on hydroxide-ion concentration, with activation energies between 13 and 15 kcal./mole. Using TLC and GLC, he found no compounds other than the expected degradation products during the reactions.

Since stability studies appearing in the literature were of a preliminary nature, providing limited kinetic information, the purpose of this study was to obtain

PGE

PGA

PGE_1: $R_1 = (CH_2)_6COOH$
$R_2 = (CH_2)_4CH_3$
PGE_2: $R_1 = CH_2CH=CH(CH_2)_3COOH$
$R_2 = (CH_2)_4CH_3$

PGB

Scheme I

additional data dealing with the relative rates of the dehydration and rearrangement reactions of both PGE_1 and PGE_2.

EXPERIMENTAL

UV Spectroscopy—An automatic recording spectrophotometer[2] afforded UV measurement at the two wavelengths of choice at definite intervals over extended periods of time. The instrument was equipped with an automatic reference compensator, a wavelength programmer, and an auxiliary dwell unit. A thermistor probe was attached to the sample compartment so that the cell temperature could be automatically recorded prior to the absorbance reading. The temperature was controlled by a constant-temperature circulator[3].

Stock solutions of prostaglandins were accurately prepared at a concentration of 1.0 mg./ml. in 90% aqueous ethanol and stored at 0°. Measurements were made on triplicate stock solutions. A 75-μl. aliquot of the stock solution was measured in a gastight syringe[4] equipped with a Teflon-tipped plunger and Chaney adaption for reproducible delivery. Four 5.0-ml. quantities of aqueous solvent, measured in volumetric flasks, were pre-equilibrated to the temperature at which the study was to be conducted. One aliquot of PGE stock solution was added to each of three solvent flasks at zero time. The fourth solution served as the reference blank. The final concentration of prostaglandin in the solutions was 4.20×10^{-5} M. Because absorbance readings were conducted at 218 nm., it was necessary to take certain precautions; for example, special cells[5] were used which have a high percent transmission below 200 nm. In addition, end absorption due to buffer species was overcome by dilution. The buffers used were sodium dihydrogen phosphate–citric acid (pH 2.2–8.0), glycine–sodium hydroxide (pH 8.6–10.6), glycine–hydrochloric acid (pH 2.2–3.6), and hydrochloric acid–potassium chloride (pH 1.1–2.2). In all but the latter system, the buffer solutions were diluted to approximately 0.002 M, while the concentration of the hydrochloric acid–potassium chloride buffer was 0.05 M in potassium chloride.

Beer's law plots were made for the aqueous PGE_1 solutions, and the values of the absorptivity of the PGA and PGB species were calculated from aqueous acid and base treatments, respectively ($\lambda_{max}PGA = 218$ nm., $\epsilon_{218} = 9500$; $\lambda_{max}PGB = 278$ nm., $\epsilon_{278} = 20,500$). The measurement of PGE could be made at any time interval during the degradation by calculating the concentrations of PGA and PGB from the absorbances at 218 and 278 nm., respectively. Overlap of the 278-nm. peaks at 218 nm., however, necessitated a correction factor f for the calculation of $A_{PGA(218)}$. This factor maintained a constant value equal to the ratio of the absorbance of PGB at 218 nm. to its absorbance at 278 nm. Therefore:

$$A_{PGA(218)} = A_{obs(218)} - f \cdot A_{PGB(278)} \quad \text{(Eq. 1)}$$

where $f = 0.135$. Then:

$$X_E + X_A + X_B = 1 \quad \text{(Eq. 2)}$$

and:

$$X_E = 1 - \frac{A_{PGA(218)}}{\epsilon_{218} \cdot E_i} - \frac{A_{PGB(278)}}{\epsilon_{278} \cdot E_i} \quad \text{(Eq. 3)}$$

where E_i = initial molar concentration of E, and X represents mole fraction.

The rate constant k_1 in the degradation sequence (Scheme II)

$$\text{PGE} \xrightarrow{k_1} \text{PGA} \xrightarrow{k_2} \text{PGB}$$

Scheme II

was accurately measured by plotting log X_E versus time, where $k_1 = -2.303 \cdot$ (slope), or by plotting $\log(X_{A\infty} - X_{A_t})$ versus time (only in the case where $k_2 \simeq 0$). The rate constant k_2 was estimated by plotting $\log(X_{B\infty} - X_{B_t})$ versus time in the region toward the end of the reaction.
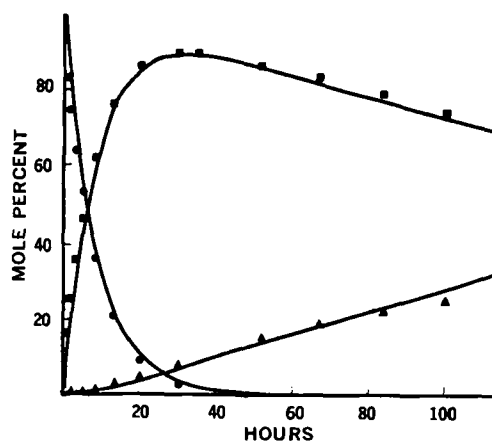
[2] Gilford model 2400-S.
[3] Braun Thermomix II.
[4] Hamilton.
[5] Suprasil.

Figure 1—*Reaction profile for $PGE_1 \rightarrow PGA_1 \rightarrow PGB_1$ at pH 8.0 and 60°. Key: ●, PGE_1; ■, PGA_1; and ▲, PGB_1.*

Since the direct measure of B contained the least error, these data points and other required parameters were entered into a computer[6]. By using a consecutive first-order program, the graphically calculated k_1 and k_2 values were used to compute the molar ratios of E, A, and B as a function of time, where:

$$E = E_i e^{-k_1 t} \quad \text{(Eq. 4)}$$

$$A = E_i k_1 (e^{-k_1 t} - e^{-k_2 t})/(k_2 - k_1) \quad \text{(Eq. 5)}$$

and:

$$B = E_i[1 - (k_1 e^{-k_2 t} - k_2 e^{-k_1 t})/(k_1 - k_2)] \quad \text{(Eq. 6)}$$

The average absolute deviation from the observed values was used as a criterion of accuracy for the computed values. The estimated rate constants were varied by an iteration procedure so as to minimize this deviation. The "best fit" values thus obtained were considered to be closer to the true values than were the experimentally observed data calculated from Eqs. 1–3.

TLC—TLC provided a means of checking the initial purity of prostaglandin samples as well as monitoring samples subjected to induced degradation. It also allowed the direct study of PGE's that are transparent to UV light. The chromatographic procedures utilized were extracted from a study of analytical and preparative chromatographic methods for monounsaturated prostaglandins by Andersen (2).

Samples of prostaglandin were prepared for TLC by dissolving PGE in 90% aqueous ethanol at a concentration of 1 mg./ml. Thin-layer plates[7], 15.2 × 20.3 cm. (6 × 8 in.), coated with silica gel GF, were developed in the usual ascending manner following a partial pass in absolute methanol [to approximately 2.54 cm. (1 in.) above the origin] to pull the prostaglandins away from strong base which, if present, interfered with spot mobility. The solvent system used to resolve the parent prostaglandins apart from their degradation products was ethyl acetate–88% formic acid (400:5), and the detection methods were a 5% ethanolic solution of phosphomolybdic acid and 254 nm. UV light.

RESULTS AND DISCUSSION

In the present study the degradation behavior of the two E prostaglandins was investigated under identical buffer conditions covering a wide pH range. A temperature of 60° was chosen to accelerate the decomposition rates so that their measurement would be kinetically convenient. It was assumed that the increased rates were mediated only by thermal phenomena rather than by free radical processes initiated by light or the presence of oxygen.

Since the reaction pathway is of the first-order consecutive type above pH 4, typical concentration versus time curves were observed. In Figs. 1 and 2, the change in mole percents of the relevant species with time is shown for the E_1 and E_2 reaction profiles at 60° in pH

[6] Digital PDP-10.
[7] Analtech, Inc.
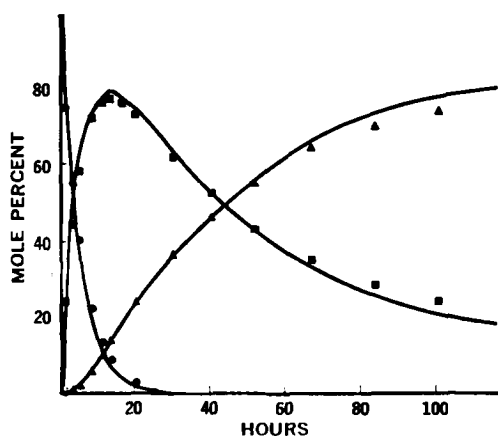
Figure 2—*Reaction profile for $PGE_1 \rightarrow PGA_1 \rightarrow PGB_1$ at pH 8.0 and 60°. Key: ●, PGE_1; ■, PGA_1; and ▲, PGB_1.*

8.0 buffer, respectively. These profiles suggest that the concentration of the original β-hydroxyketone moiety (E) decreased by a first-order rate and that E_2 decreased faster than E_1. The intermediate α,β-unsaturated ketone product (A) rose to a maximum after about 13 hr. for E_2 and after 32 hr. for E_1. In addition, the height of the maximum for A_2 was less than that of A_1 (79% versus 89%). After this maximum was reached, the A products decreased in an approximately linear fashion. Following an induction period (3 hr. for B_2 and 10 hr. for B_1), the concentration of the rearranged thermodynamically more stable product (B) rose rapidly and tailed off upon approaching completion of the consecutive reaction. Although mechanistic theory dictates these reactions to be reversible, experimental and resulting thermodynamic considerations showed the reaction to favor overwhelmingly formation of the PGB compounds under all testing conditions, resulting in negligible reverse reaction rate constants.

The initial content of the intact E compound only approached the theoretical concentration of 100%. This was possibly due to a small amount of A compound initially present in the stock solutions. These stock solutions, although stored at zero degrees, showed minor degradation after 1 month. When necessary to obtain an approximate "zero time" at which 100% E was present, the plots were extrapolated to 0% A and 0% B on an appropriately adjusted time axis.

Below pH 4 the concentration of E approached a final value asymptotically as the reaction proceeded toward completion. At the pH levels investigated between 1 and 4, the reaction of A → B became insignificant, while the reaction E → A proceeded by a pseudo-first-order rate law since the solvent (water) was present in large excess. At very high acid concentrations, degradation continued beyond the formation of A, as shown in Scheme III, where X was an unidentified product(s) that formed from either E_1 or E_2 and exhibited a molar absorptivity of approximately 20,000 at a λ_{max} of 325–330 nm. $(R_f \approx 0.8)$. Under the conditions of the experiments reported here, $k_1 \gg k_2$, for which reason the A → X reaction was neglected in the kinetic treatment of the E → A reaction. When the dehydration step was treated as a first-order reaction, the values of k_1 could be calculated from the slope of the graph represented by Fig. 3. The reaction was also first order with respect to prostaglandin since little variation in the rate constants was observed upon varying initial reactant concentration.

Above pH 4, however, the rearrangement reaction became significant[8]. In the general consecutive reaction (Scheme II), the E compo-

$$E \xrightarrow{k_1} A \xrightarrow{k_2} X$$

Scheme III
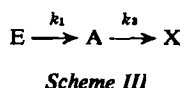
[8] Some ester formation could have occurred at the free carboxyl group due to the presence of a small quantity of ethanol in the sample from the stock solution. For present purposes, this esterification was considered to exert an insignificant effect on the rates of dehydration and rearrangement reactions. The occurrence of ester formation was observed on thin-layer chromatograms where the R_f of the ester slightly exceeded that of its corresponding acid.
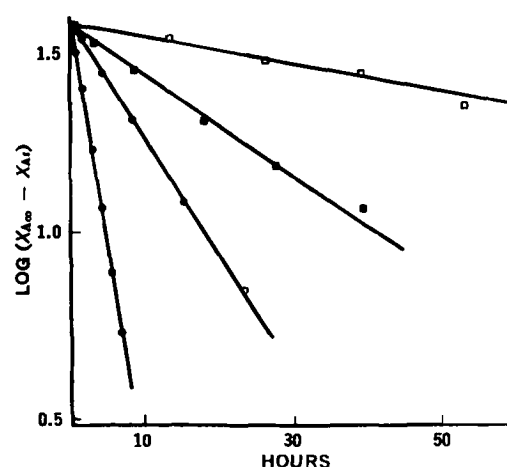
Figure 3—*Dehydration of PGE in acid solutions at 60°. Key: ●, pH 1.22; ○, pH 1.81; ■, pH 2.20; and □, pH 3.01.*

nent represented the only component that could be described by a simple first-order equation (Eq. 4). Both the A and B components were biexponential functions which had a time course that was dependent upon both k_1 and k_2 (Eqs. 5 and 6). Data representing A or B could not be analyzed for either k_1 or k_2 by the usual first-order plotting techniques. In general, over the pH range studied the rate of rearrangement was exceeded by the dehydration reaction only by an average factor of 10. While the loss of E was dependent on k_1, the formation of A and B was dependent upon both k_1 and k_2. Thus the overall rate process was not governed by a single constant. For this reason, data collected for A and B as a function of time were first treated graphically to obtain approximate values of k_1 and k_2. These values and other required parameters were then treated by numerical integration on a digital computer to fit calculated values to the observed values of the appearance of B. These values of B were considered to be the most accurate data because of the high sensitivity of the absorbance readings (large absorptivity). The values of A at 218 nm. were frequently subject to interference from end absorption of buffer species present which required a high slit width compensation, contributing greater error to the measurement of A. When the computer-optimized values were plotted as a function of pH, characteristic log rate–pH profiles developed (Figs. 4 and 5).

The log k_1–pH profiles for the dehydration reactions of E to A are shown in Fig. 4. The pH of maximum stability in aqueous solutions of E_1 and E_2 was about 3–4. Specific hydrogen-ion catalysis (slope = −1) was evident below pH 3.0 for both E_1 and E_2. The pH-independent region of pH 3–4 probably resulted from solvent catalysis. This pH–rate profile represents the comparative rates observed at identical buffer concentrations less than 0.002 M (except at pH < 2.5 where buffer concentration reached 0.05 M). At higher buffer concentrations, a study of ionic strength effects
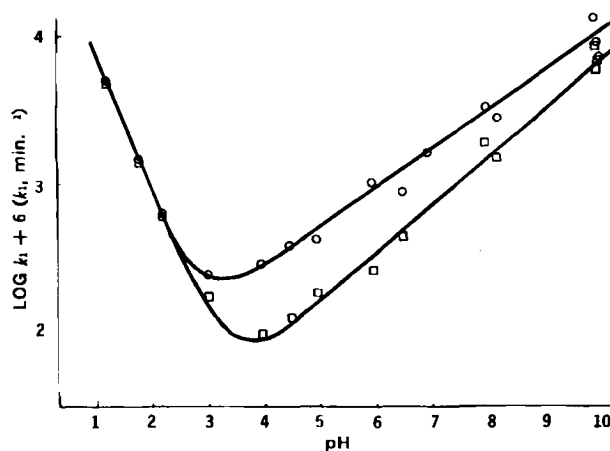

Figure 4—*Log k_1–pH profile for the dehydration of PGE_1 (□) and PGE_2 (○) at 60°.*

Table I—Thermodynamic Activation Parameters[a] for Prostaglandins E_1 and E_2

| Reaction Type | pH | E_1 | | E_2 | |
		E_a, kcal./mole	$\Delta S^{\ddagger}_{298}$, e.u.	E_a, kcal./mole	$\Delta S^{\ddagger}_{298}$, e.u.
Dehydration	1.2	18	−22.4	18	−22.4
Dehydration	8	24	−8.4	24	−7.0
Isomerization	8	20	−25.8	20	−22.3

[a] These are "apparent" values calculated from reaction in dilute aqueous buffer.

was undertaken. Only a minimal effect was noted, however, presumably due to overriding buffer catalysis. No experiments have yet been conducted to extrapolate k values to zero buffer concentration. Therefore, although specific hydroxyl-ion catalysis contributed most significantly to dehydration in alkaline conditions, general acid–base (solvent) catalysis at moderate pH's decreased the slope of the alkaline branch of the catenary to less than +1 (slope ~ 0.3). The difference in values of the rate constants for the dehydration of E_1 and E_2 under these solvent conditions was evident at alkaline pH's. In general, E_2 dehydrated twice as fast as E_1. Sufficient data were not available at this time to determine if the slopes of the lines were actually parallel. Although the absolute values of k_1 were significantly different, this difference appeared to be relatively independent of pH. At pH 9.0, as in acid media, the rates of reaction were proportional to the initial concentration of reactant, again suggesting first-order dependence on E concentration.

The log k_2–pH profiles for the rearrangement reactions of E_1 and E_2 are represented by the linear graphs shown in Fig. 5. The slopes of these lines were again $< +1$ (0.5 for E_1 and 0.35 for E_2), suggesting that more than simple specific hydroxyl-ion catalysis was involved. Below pH 7.0, the rate of the rearrangement reaction for E_1 became negligible, whereas the rearrangement of E_2 remained significant down to pH 5. In the case of k_2 the relationships of rate and pH between the two E prostaglandins in dilute aqueous buffer were dissimilar. The rate of E_2 rearrangement was significantly faster than E_1 at intermediate pH's, while at high pH their rates became comparable.

From this discussion, no definite conclusions could be reached with respect to the specific mechanisms involved in the reaction. Since k_1 was greater than k_2 only by an order of about 10 over a broad range of pH, the isomerization step could not be considered significantly rate determining. The values of the thermodynamic activation parameters derived from the Arrhenius plot (Fig. 6), listed in Table I, allow some general insight into the mechanistic differences between E_1 and E_2. The differences in their thermodynamic character are attributable to conformational properties that affect molecular orientation during both dehydration and isomerization. Nevertheless, it is difficult to propose a full explanation for the shapes of the reaction profiles at this time.

For the acid-catalyzed dehydration reaction, the slopes of the log rate–pH profiles for E_1 and E_2 were identical (Fig. 4). Furthermore, the rate constants (k_1) under the same conditions were also identical,
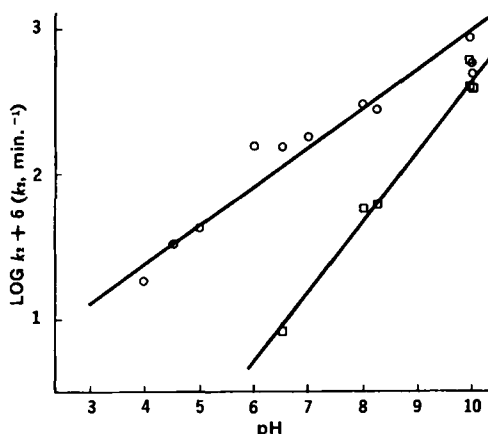
which implied a similar mechanism of dehydration for both compounds under the given acid conditions. Any intramolecular effect of the carboxyl group on this reaction was presumed negligible because of its lack of physical proximity to the reaction center. Although E_2 is slightly more acidic than E_1 [pKa(E_2) = 5.32, pKa(E_1) = 5.70 as measured in 85% aqueous ethanol], this difference in acidity was considered inconsequential with respect to its influence on reaction rates. Intermolecular participation of the acid moiety also appeared insignificant since studies yielded an invariant rate constant over a moderate range of substrate concentration. The alkaline side of the catenary·for the k_1 profile was slightly different for E_1 and E_2. The slopes of their reaction profiles approached the same magnitude, suggesting a constant ratio of k_1 (E_1) to k_1 (E_2), the value of which was approximately 2.5. The apparent energy of activation for E_1 was similar to that of E_2 in the given solvent environment, while their apparent entropies of activation were slightly different. This difference in entropy can be explained by the orientation requirements of solvent molecules around the elimination reaction centers of the two compounds. It is expected that their mechanisms of base-catalyzed dehydration are similar to one another.

In contrast, the slopes in the log rate–pH profile for the rearrangement reactions were markedly different for E_1 and E_2 (Fig. 5). This reaction has two primary driving forces: (a) the tetrasubstituted PGB double bond is more stable than that of PGA (6), and (b) it contains a system of extended conjugation. The only structural difference between the two molecules is the cis-Δ^5 double bond of E_2, which occurs as a saturated bond in E_1. Rotation about the 7–8 carbon–carbon bond could place this double bond of E_2 in a position such that it could interact with the ketone moiety at position 9. Such an interaction, however, would influence the relative reaction rates in the opposite direction to that observed, i.e., inhibit rather than accelerate the E_2 rearrangement with respect to E_1. The apparent minimum energy differences between the reactants and their transition states were identical for both molecules. The apparent

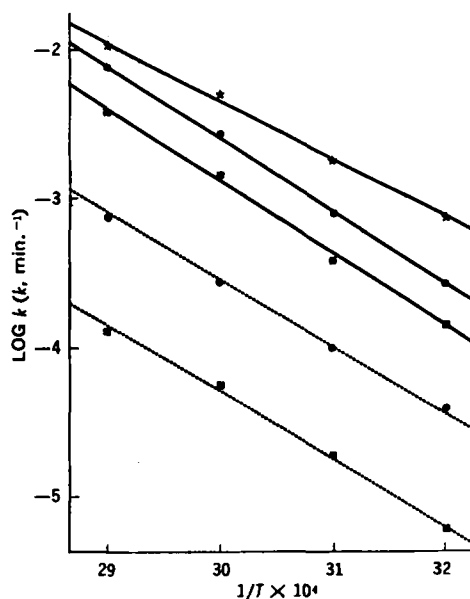Figure 5—Log k_2–pH profile for the rearrangement of PGA₁ (□) and PGA₂ (○) at 60°.

Figure 6—Arrhenius plot of log k against 1/T showing temperature dependence of rates of dehydration and rearrangement of PGE₁ and PGE₂. Key: ■, PGE₁; ●, PGE₂ at pH 8.0; ★, PGE₁ and PGE₂ at pH 1.2; ———, k₁; and - - -, k₂.

entropy of activation values for E_1 and E_2 were different, however, which suggested an explanation for the difference in reaction rates. The large negative values of $\Delta S^{\ddagger}$ reflect a significant requirement in molecular orientation, presumably of the side chains. The cis-Δ^5 double bond imposes certain restraints on the free rotation (lowering its degree of freedom) of the C-8 side chain vis-á-vis the C-12 side chain, causing increased nonbonded interactions when both chains lie side by side. These factors become especially important when a carbanion is introduced into the cyclopentenone ring at C-12, forcing the associated side chain and ring to lie flat in one plane. Some of these interactions are relieved if the unsaturation in the C-8 side chain is removed. It then has a more "floppy" nature, which allows it to lie away from the now rigid C-12 side chain. Thus, E_2 contains a lower absolute entropy in its reactant state than E_1, which must accordingly suffer a greater entropy loss during conversion to its carbanion transition state. These interpretations hold only qualitative value since absolute thermodynamic parameters must be determined only at zero buffer concentrations. Nevertheless, the data presented here retain comparative significance and indicate that the difference in behavior between E_1 and E_2 is clearly the result of complicated conformational effects.

In summary, there is a difference in the rates of dehydration and rearrangement between E_1 and E_2. No well-defined mechanism illustrating the reason for this difference can be presented at this time;

however, such a mechanism should involve participation of the cis-Δ^5 double bond which is present only in E_2.

REFERENCES

(1) S. M. M. Karim, J. Devlin, and K. Hillier, *Eur. J. Pharmacol.*, **4**, 416(1968).
(2) N. H. Andersen, *J. Lipid Res.*, **10**, 320(1969).
(3) H. C. Brummer, *J. Pharm. Pharmacol.*, **23**, 804(1971).
(4) J. E. Shaw and P. W. Ramwell, *Methods Biochem. Anal.*, **17**, 325(1969).
(5) T. O. Oesterling, paper presented to the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Washington meeting, 1970.
(6) G. Stork, G. L. Nelson, F. Rouessac, and O. Gringore, *J. Amer. Chem. Soc.*, **93**, 3091(1971).

Metabolism of the Antihypertensive Agent 1,4-Dihydro-2,6-dimethyl-4-(2-trifluoromethylphenyl)-3,5-pyridinedicarboxylic Acid Diethyl Ester

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Abstract ☐ The absorption, distribution, metabolism, and excretion of 1,4-dihydro-2,6-dimethyl-4-(2-trifluoromethylphenyl)-3,5-pyridinedicarboxylic acid diethyl ester, an antihypertensive drug, was studied in rat, dog, and squirrel monkey. The drug, labeled with ^{14}C, was found to undergo aromatization, hydrolysis of its ester groups, and oxidation of a ring methyl and an ethyl group. No dihydropyridyl metabolites or the hypothetical aromatized parent compound were found. The metabolites identified were: 2,6 - dimethyl - 4 - (2 - trifluoromethylphenyl) - 3,5 - pyridinedicarboxylic acid, its monoethyl and mono-2-hydroxyethyl esters, and 2-hydroxymethyl - 6 - methyl - 4 - (2 - trifluoromethylphenyl) - 3,5-pyridinedicarboxylic acid lactone. The form in which the drug was administered was found to have a profound effect on its absorption.

The crystalline powder administered to dogs in gelatin capsules was poorly absorbed; solubilization with polyethylene glycol 200 resulted in a fivefold increase in absorption.

Keyphrases ☐ 1,4-Dihydro-2,6-dimethyl-4-(2-trifluoromethylphenyl)-3,5-pyridinedicarboxylic acid diethyl ester, radiolabeled—absorption, distribution, metabolism, and excretion in rats, dogs, and monkeys ☐ Antihypertensive agents—metabolism of 1,4-dihydro - 2,6 - dimethyl - 4 - (2 - trifluoromethylphenyl) - 3,5 - pyridinedicarboxylic acid diethyl ester, in rats, dogs, and monkeys ☐ Absorption — 1,4 - dihydro - 2,6 - dimethyl - 4 - (2 - trifluoromethylphenyl)-3,5-pyridinedicarboxylic acid diethyl ester, in rats, dogs, and monkeys

1,4-Dihydro-2,6-dimethyl-4-(2-trifluoromethylphenyl)-3,5-pyridinedicarboxylic acid diethyl ester[1] (I) is an effective agent for lowering blood pressure by direct vascular dilatation in normotensive, neurogenic, and renal hypertensive dogs. Hypotensive effects were also observed in rats, rabbits, and guinea pigs. The drug also lowered blood pressure in human subjects at relatively low oral doses (10 mg.).

As part of the preformulation and preclinical investigation of this compound, its absorption, distribution, metabolism, and excretion in rats, dogs, and monkeys were studied. These studies were facilitated by the use of the ^{14}C-labeled drug.

EXPERIMENTAL

Synthesis of Labeled Drug—Compound I-4-^{14}C was prepared as shown in Scheme I. ^{14}C-Labeled drug was shown to be free of radiochemical impurity and had a specific activity of 4 µc./mg.

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[1] SK & F 24260.