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Research Article

Stability of Prostacyclin Analogues: An Unusual Lack of Reactivity in Acid-Catalyzed Alkene Hydration[1]

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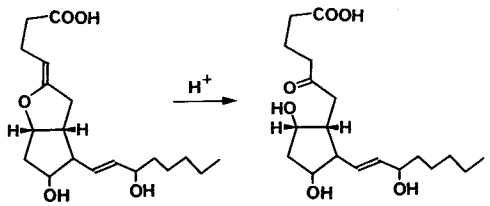
Prostacyclin analogue 5 undergoes specific acid-catalyzed hydration ($k_{H^+} = 1.9 \times 10^{-7} M^{-1}$ sec^{-1} at 25°C) and a pH-independent oxidation reaction ($k_0 = 1.2 \times 10^{-10}$ sec^{-1} at 25°C) above pH ~5. The hydration reaction for 5 is much slower than for other structurally similar exocyclic alkenes, even though the rate-determining step is proton transfer. This slowness of reaction and an analysis of the pH–rate profile show that 5 does not exhibit significant intramolecular general acid catalysis, as does prostacyclin.

KEY WORDS: prostacyclin analogue degradation; lactonization; alkene hydration; pH effect; proton transfer.

INTRODUCTION

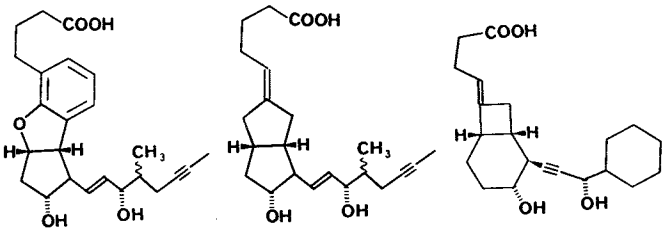
Prostacyclin (epoprostanol; PGI_2) (1) is a potent naturally occurring inhibitor of platelet aggregation that has been used as a therapeutic agent for heart attack and stroke (1). Prostacyclin has also been used occasionally in antithrombotic treatments (2), hemoperfusion (3), certain peripheral vascular disorders (4–6), and cardiopulmonary bypass surgery (7). Unfortunately, the usefulness of prostacyclin as a widespread therapeutic agent is limited because of its chemical instability—it has a lifetime at physiological pH of only ≃3 min. This reactivity is due to the vinyl ether group, which undergoes facile acid-catalyzed hydrolysis to give 2, even in the neutral pH region (Scheme I).



1 Scheme I 2

Further, prostacyclin reacts ≃100 times faster than expected based on the reactivity of other vinyl ethers (8). The mechanism for this enhanced rate of reaction was not determined in earlier studies, however, it was deduced to involve the carboxyl group (9). Recently, Kresge and colleagues have provided compelling evidence using fractionation factor analysis to show that this rate enhancement is due to general acid catalysis by the carboxyl group (10). At physiological pH, this group exists largely in the carboxylate form; a small fraction, however, is in the highly reactive protonated form (10,11).

The finding that prostacyclin is very susceptible to intramolecular carboxyl catalysis may have direct consequences on the stability of prostacyclin analogues. Although several of the new analogues such as 3 (TRK-100), 4 (iloprost) (13,14), and 5 (RS-93427) (15–17) (Scheme II) are not



3 4 5

Scheme II

vinyl ethers and so are not subject to hydrolysis, some still have a 4,5 or 5,6, double bond which may be susceptible to intramolecular acid-catalyzed hydration (18,19). To probe the mechanism(s) of alkene hydration of prostacyclin analogues containing an exocyclic double bond and a remote carboxyl group, we studied the degradation of antithrombotic compound 5 in aqueous solution.

EXPERIMENTAL

Materials. Prostacyclin analogue 5 was prepared by the Institute of Organic Chemistry at Syntex Research (15–17). Citric acid, acetic acid, KH_2PO_4, H_3PO_4, H_2SO_4, NaOH, D_2O, HCl, DCl, and NaCl were reagent grade (Aldrich or Mallinckrodt) and were used without further purification. The mobile phase was prepared from high-performance liquid chromatography (HPLC)-grade acetonitrile (Burdick and Jackson) and distilled deionized water.

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Synthesis. The lactone diastereomers (7) were prepared as follows: a mixture of the calcium salt of 5 (90 mg, 0.123 mmol) in 10 ml of 2 *N* H_2SO_4-CH_3CN (1:1) was stirred at 75–80°C for 4.5 days. The reaction mixture was diluted with water and extracted with ethyl acetate (3×). The combined extracts were washed with saturated sodium bicarbonate solution. After drying over sodium sulfate and evaporation of the solvent, the residue was chromatographed on silica gel using ethyl acetate–hexane (7:3) to yield 4-{(3′S,1S,2S,3R,6S,7SR)-2-(3′-cyclohexyl-3′-hydroxyprop-1′ - ynyl) - 3,7 - dihydroxybicyclo[4.2.0]oct - 7 - yl}butyric acid 1,5-lactone (7) as its less polar (8 mg, 10%) and more polar (12 mg, 15%) diastereomers. The data for the less polar diastereomer were as follows: ^{1}H NMR, δ = 0.9–2.8(m,28), 3.6(m,1,H-11), 4.16(t,1,J = 7.5,H-15); IR(CH_2Cl_2) 1730 cm^{-1} (C=0); high-resolution mass spectra, calculated for $C_{21}H_{28}O_3$(M-H_2O): 328.20385, found: 328.20383. The data for the more polar diastereomer were as follows: ^{1}H NMR, δ= 0.95–2.85(m,28), 3.53(m,1,H-11), 4.18(m,1,H-15); IR(CH_2Cl_2) 1720 cm^{-1} (C=0); high-resolution mass spectrum, calculated for $C_{21}H_{28}O_3$(M-H_2O): 328.20385, found: 328.20416.

4-Cyclobutylidene butyric acid (9) was prepared as follows: to a solution of 3-ethoxycarbonylpropyltriphenyl phosphonium bromide (1.52 g, 3.33 mmol) in 5 ml of tetrahydrofuran was added potassium *t*-butoxide (0.34 g, 3.03 mmol). The dark orange solution was stirred for 0.5 hr and cyclobutanone (0.1 g, 1.52 mmol) was added. The reaction mixture was stirred at 23°C for 1.5 hr and then poured into water. The mixture was extracted with ethyl acetate (2×) and the combined extracts were washed with brine. After drying over sodium sulfate and evaporation of the solvent, the residue was chromatographed on silica gel using ethyl acetate–hexane (1:9) to afford the esterified product. The ester was cleaved by stirring 90 mg of the ester (0.54 mmol) and 2.7 ml of 0.5 *N* LiOH (1.34 mmol) in 3 ml of methanol at 23°C for 16 hr. The reaction mixture was acidified with dilute HCl and extracted with ethyl acetate (3×). The combined extracts were washed with brine and dried over sodium sulfate. After evaporation of the solvent, the residue was chromatographed on silica gel using CH_2Cl_2–CH_3OH–HOAc (94.9:5.0:0.1) to give 9 (70 mg, 93%). ^{1}H NMR, δ = 1.6–2.8(m,10), 5.0(m,1,H-4), 9.0–9.7(br s,1,COOH); high-resolution mass spectrum, calculated for $C_8H_{12}O_2(M^+)$: 140.08373, found: 140.08418.

Apparatus. The kinetic analysis of 5 and its degradation products was carried out using an HPLC system consisting of a Micromeritics Model 725 autoinjector, Model 110A Altex pump, Model 770 Kratos spectrophotometric detector, and SP 4000 computing integrator. The following reversed-phase (RP)-HPLC conditions provided a linear response throughout the range of 0.01–10 μg injected: column, 250 × 4.6-mm Phenomenex Partisil 5 ODS 3; mobile phase, 55% (v/v) H_2O (with 0.5 ml/liter added CH_3COOH)/45% (v/v) CH_3CN; flow, 1 ml/min; detection, 0.08–0.64 AUFS at λ = 210 nm; injection volume, 20 μl; and typical retention times, 10 min for 5 and 15 min for the 15-keto product, 8. Under similar conditions, 9 gave a retention time of 9 min. Collection of the degradation products was carried out using an LKB Multi-Rac fraction collector. UV spectra of the major RP-HPLC peaks were obtained by monitoring the HPLC effluent using an HP-8450A diode-array spectrophotometer equipped with an 8-μl flow cell. The pH's of the reaction solutions were determined immediately after preparation using a Radiometer PHM 64 pH meter and Model GK2401C combination electrode. Gas chromatographic separation of 5 from the lactone diastereoisomers (7) was carried out using a Varian 3700 gas chromatograph and the following conditions: column, 15-m × 0.32-mm DBl; oven, 100°C for 2 min followed by a 15°/min gradient to 280°C; carrier gas, 1.0 kg/cm^2 He; injector, 250°C; and retention times, 210 sec for 5, 217 sec for 7a, and 225 sec for 7b. Electron impact mass spectra (EI-MS) were obtained using a Finnigan MAT 112S direct inlet mass spectrometer; chemical ionization mass spectra (CI-MS) were obtained using NH_3 as the reagent gas. High-resolution mass spectra were obtained using a Finnigan-MAT 311A. ^{1}H-NMR spectra were measured on either a Bruker WM 300 (300-MHz) or an EM-390 (90-MHz) instrument. Spectra were determined in $CDCl_3$ solutions and were referenced to internal tetramethylsilane; coupling constants are given as hertz.

Kinetics. In order to obtain pseudo first-order kinetics, the buffer concentration was always maintained in excess over the drug concentration (≃1.5 × 10^{-4} *M*). The total buffer concentration was usually 0.01 *M* and the pH of each solution containing 5 was determined at the reaction temperature. Ionic strength was held constant at 0.15 *M* by the addition of sodium chloride. Buffer dilution studies were carried out by measuring the degradation kinetics in solutions of various buffer concentration at a constant buffer ratio and ionic strength. In a typical experiment, 5-ml aliquots of reaction solution containing 0.1 mg/ml of the bis-calcium salt of 5 were transferred to pretreated clear ampoules, flame-sealed, and stored at 60, 80, and 100°C. At known time intervals, ampoules were removed from the constant-temperature oven and refrigerated. Upon removal of the last sample, the stored samples were then all analyzed on the same day along with a refrigerated control. Usually, 8–12 samples per kinetic run were collected, and the peak area integrations were converted to concentrations or percentage remaining values by the use of linear response calibration curves determined for a solution of a known concentration of 5.

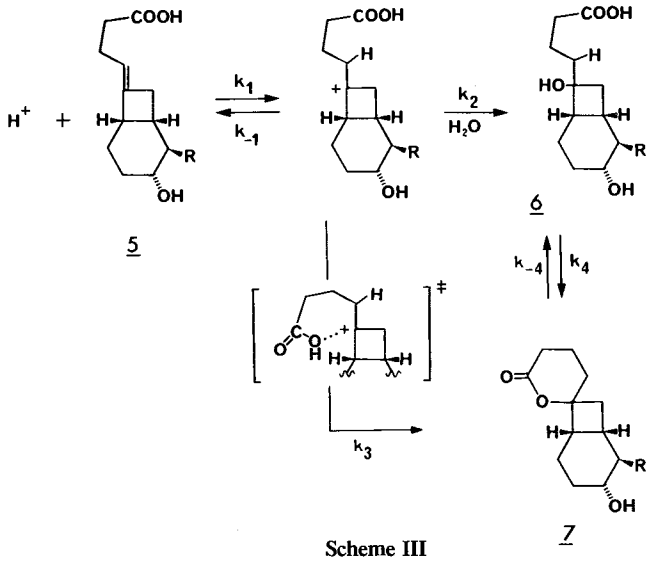
Product Identification. The lactone diastereomers (7) were isolated on a preparative scale from a reaction mixture of 5 and 0.1 *M* HCl after reaction for one half-life at 80°C or with 2N H_2SO_4-CH_3CN (1:1) at 75°C for 4.5 days. The diasteromeric lactones were separated into individual stereoisomers either by chromatography on silica gel or by gas chromatography. Gas chromatographic analysis showed that, under acid conditions, the major products were lactone diastereomers (7). Identity of the lactone diastereomers was made by NMR and mass spectral analysis of isolated reaction product. The 15-keto oxidation product (8) was isolated by semi-preparative reversed-phase (RP) HPLC from a sample of 5 allowed to react in the solid state at 50°C for 2 weeks. Under these reaction conditions, 8 was the major product. The structure of the 15-keto compound (8) was confirmed using electron impact and chemical ionization mass spectrometry and by ^{1}H-NMR spectrometry. The ^{1}H-NMR spectrum of 8 lacks the downfield resonance at 4.15 ppm present in 5 (assigned as the 15β-H). The 12α-H reso-

nance at 2.56 ppm in 8 is a clean triplet due to couplings with 7β-H and 11β-H, whereas in 5 it appears as a doublet of triplets due to the additional coupling across the triple bond with 15β-H. The rest of the ^{1}H-NMR spectrum of 8 is similar to that of 5 (15–17). The EI-MS of 8 showed a molecular ion at *m/z* 344 and two subsequent losses of water (*m/z* 326,308) as well as loss of the cyclohexyl moiety (*m/z* 261). Chemical ionization (CI) mass spectrometry using ammonia as the reagent gas showed intense MH^+ and MNH_4^+ signals at *m/z* 345 and 362.

Isotope Incorporation Analysis. A solution of 5 (0.08 mg/ml) in 0.0115 *M* DCl was divided into several 10-ml aliquots and flame-sealed in clear glass ampoules. Some of these were heated at 80°C for known time intervals; others were held at RT as controls. After approximately one half-life, the solutions were extracted with toluene and dichloromethane and dried with anhydrous $MgSO_4$, and the organic solvents removed by rotary evaporation. The remaining starting material was separated and analyzed by gas chromatography/mass spectrometry (GC/MS) using the conditions described under Apparatus.

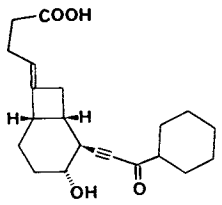
RESULTS AND DISCUSSION

Reaction Products. In acid, 5 undergoes loss of the 4,5 double bond to give a mixture of the lactone diastereomers (7). Two reaction pathways are likely: (i) the carbonium ion intermediate may be quenched by water (k_2), giving the alcohol product 6 (reaction in top row in Scheme III), followed by lactonization (k_4) to give the diastereomeric lactones (7); or (ii) the carboxyl moiety may react directly with the carbonium ion (k_3), affording 7 without the alcohol intermediate (reaction in bottom row in Scheme III). The choice of pathway is governed by the kinetics of the carbonium ion quenching reaction. Most carbonium ions are extremely reactive and, as such, show little selectivity; they react most rapidly with the nucleophile of greatest concentration. A crude estimate of the relative water and carboxyl concentrations can be made by comparing the concentration of water in water (55 *M*) with the effective molarity, or EM, of the neighboring carboxyl group. Although the EM for reaction of a carboxyl group with a carbonium ion is unknown, an upper limit may be estimated from the EM for lactonization of γ- or δ-hydroxy carboxylic acids. The EM for γ-hydroxy butyric acid is 80 (20); for δ-hydroxy valeric acid the EM is approximately twice as large (21). Comparison of these EMs with 55 *M* for water suggests that the carbonium ion of 5 is quenched by *both* pathways, namely, k_2 and k_3. The lactone diastereomers are observed as the final products because k_4 and k_{-4} are significantly larger than k_1 and because the k_4 equilibrium between 6 and 7 favors the lactone ($k_4 > k_{-4}$) (20).



Scheme III

In the neutral pH region, the major degradation mechanism is oxidation, not hydration. Evidence for this comes from the variation in observed rate constants above pH 5. The irreproducibility in these rate constants arises because the samples have different amounts of oxygen present and therefore show different rates of oxidation. For example, at pH 8.1 and 100°C, 5 reacted approximately four times faster under O_2 than when freeze-thaw degassed to remove all traces of O_2. Further evidence that 5 is susceptible to oxidation at a neutral pH is shown by the effect of the oxidants H_2O_2 and $Fe(CN)_6^{3-}$ on the reaction rate. The addition of 0.035 *M* H_2O_2 to a solution of 5 at pH 8 and 80°C increased the degradation rate ~240-fold; the addition of 0.01 *M* $K_3Fe(CN)_6$ under similar reaction conditions gave a 30-fold increase. The product profile at neutral pH's is not yet fully understood, however, one of the minor oxidative degradation products demonstrated the same HPLC retention time as 8, the solid-state degradation product (Scheme IV).



Scheme IV

Effect of pH and Temperature. The degradation of prostacyclin analogue 5 showed apparent first-order kinetics at all pH's. In the acid region, most reactions were followed for more than two half-lives and, occasionally, up to five half-lives. Good pseudo first-order kinetics were also obtained at these longer reaction times. In solutions of a neutral pH, the degradation was much slower and so reactions were generally followed to 50–90% drug remaining. The hydrolysis rates of 5 were determined at several temperatures (60, 80, and 100°C) and pH's (pH 1 to 12) at 0.15 *M* ionic strength (Table I). It was necessary to use a constant ionic strength because the reaction rate increased linearly with ionic strength (μ); for example, the degradation of 5 at pH 3.8 and 80°C obeyed the relationship, $k_{H^+} = [(5.8 \pm 2.0) + \mu\ (6.9 \pm 2.9)] \times 10^{-8}$ sec^{-1}. Reaction of 5 carried out at 80°C in pH 4.6 acetate buffer solutions of varying buffer concentrations (0.03 → 0.15 *M*) showed no general buffer catalysis. Citrate buffer showed slight catalysis, albeit so weak that the rate constant determined at 0.01 *M* (as done in Table I) was not statistically different from that obtained from the *y* intercept of a k_{obs} vs buffer concentration plot.

Table I. Observed Pseudo First-Order Rate Constants for the Degradation of 5 in Aqueous Solution at 60–100°C

60°C		80°C		100°C	
pH[a]	$10^8 k_{obs}$ (sec^{-1})	pH[a]	$10^8 k_{obs}$ (sec^{-1})	pH[a]	$10^7 k_{obs}$ (sec^{-1})
1.11	180 ± 4[b]	0.45	8300 ± 500	1.12	1140 ± 40
2.06	21 ± 1[c]	1.12	1340 ± 20[d]	2.06	155 ± 7
3.80	0.94 ± 0.06	2.06	164 ± 5[e]	3.90	5.5 ± 0.3
4.37	1.20 ± 0.02	3.85	4.4 ± 0.3	4.45	5.8 ± 0.2
5.37	1.3 ± 0.1	4.41	6.8 ± 0.3	5.50	2.8 ± 0.2
5.96	1.4 ± 0.1	5.43	2.7 ± 0.3	6.05	7.1 ± 0.2
6.94	1.70 ± 0.05	6.01	9.1 ± 0.2	7.07	7.0 ± 0.2
7.86	1.3 ± 0.2	6.99	13.6 ± 0.3	11.08	7.3 ± 0.4
9.56	0.92 ± 0.19	9.29	2.2 ± 0.2		
10.80	0.38 ± 0.12	10.23	1.2 ± 0.2		
11.90	1.2 ± 0.2	11.48	6.9 ± 0.5		

[a] Below pH 3, hydrochloric acid was used as the reaction solution; above pH 10, sodium hydroxide was used. In the intermediate pH range, 0.01 *M* acetate or phosphate buffers were used to minimize the rate contribution by buffer catalysis. pH's at 100°C were determined by extrapolation from pH's determined at 25, 60, and 80°C (see text).
[b] Standard deviation.
[c] In 0.01 *M* DCl the observed rate constant was (27 ± 2) × 10^{-8} sec^{-1}.
[d] In 0.05 *M* H_2SO_4 (0.1 *M* H^+) the observed rate constant was (6.2 ± 0.4) × 10^{-6} sec^{-1}.
[e] In 0.0115 *M* DCl the observed rate constant was (260 ± 18) × 10^{-8} sec^{-1}.

The pH-rate profile for the degradation of 5 (Fig. 1) shows two distinct regions having a slope of −1 in the acid region and a broad plateau region above pH ≈5, in accord with Eq. (1).

$$k_{obs} = k_{H^+}a_{H^+} + k_o \quad (1)$$

In Eq. (1) and what follows, the rate-constant subscripts denote the catalytic species; for example, k_{H^+} and k_o are the rate constants for specific hydronium ion catalysis and a spontaneous reaction, respectively. The spontaneous reaction may be due to oxidation, hydrolysis, or a combination of both. The hydronium ion activity is given by a_{H^+} and can be converted to hydronium ion concentration using known activity coefficients (f_{H^+}) and the equation $a_{H^+} = f_{H^+}[H^+]$. It was unnecessary to account for the fractions of protonated and deprotonated substrate in order to fit the data. Figure 1 also shows that the rate behavior defined by Eq. (1) is observed over a range of temperatures. Nonlinear least-squares analysis (22) of log k_{obs} versus pH using Eq. (1) afforded the rate constants in Table II. These rate constants show linear Arrhenius behavior, giving the activation parameters for k_{H^+} and k_o in Table III.

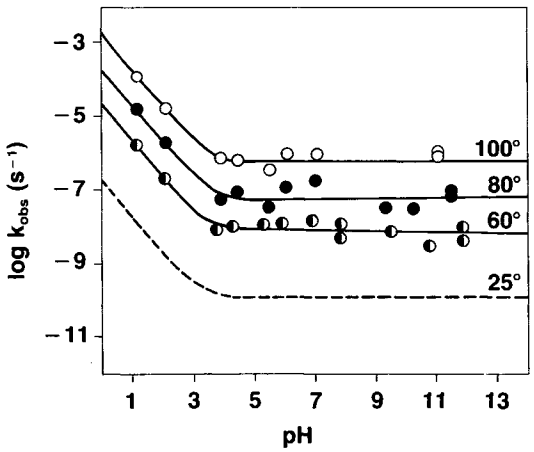


Fig. 1. pH–rate profile for the degradation of 5 in aqueous solution at 60, 80, and 100°C. The dashed line is the calculated pH–rate profile at 25°C and is from rate constants derived using the activation parameters in Table III.

Table II. Secondary Rate Constants for the Degradation of 5 in Aqueous Solution

Temp (°C)	k_{H^+} (M^{-1} sec^{-1})[a]	k_o (sec^{-1})
25[b]	1.9×10^{-7}	1.2×10^{-10}
60	(2.2 ± 0.7) × 10^{-5}	(9.2 ± 1.3) × 10^{-9}
80	(1.7 ± 0.7) × 10^{-4}	(4.3 ± 0.8) × 10^{-8}
100	(1.6 ± 0.6) × 10^{-3}	(4.3 ± 0.9) × 10^{-7}

[a] These rate constants were not corrected for solution activity; the secondary rate constants shown here were calculated using $a_{H^+} = 10^{-pH}$.
[b] Rate constants at 25°C were calculated from the 60, 80, and 100°C data using the Arrhenius equation (see Table III).

Lack of Significant Intramolecular Acid Catalysis. Prostacyclin analogue 5 was studied to determine if it is susceptible to intramolecular acid catalysis by the carboxyl group, as is prostacyclin. Reports of carboxyl group–alkene association, for example, in the selective epoxidation of arachidonic acid derivatives (23), also warn that intramolecular catalysis in 5 may occur. Intramolecular catalysis can often be detected kinetically by determining the effect of pH and acid dissociation on the reaction rate. This is shown in Eq. (2), where 5-COOH and 5-COO^- represent protonated and deprotonated 5, respectively.

$$\begin{array}{c} K_a \\ 5\text{-COOH} \rightleftarrows 5\text{-COO}^- + H^+ \\ k_{H^+}\searrow \quad \swarrow k'_{H^+} \\ \text{hydration product(s)} \end{array} \quad (2)$$

The rate law for this reaction is given by Eq. (3), which assumes that the spontaneous reaction is pH independent; i.e., k_o does not depend on K_a. If

$$k_{obs} = \frac{k_{H^+}a_{H^+}^2 + k'_{H^+}K_a a_{H^+}}{a_{H^+} + K_a} + k_o \quad (3)$$

Table III. Activation Parameters for the Degradation of 5 in Aqueous Solution

Rate constant	E_a (kcal mol^{-1})	ln A	$\Delta H^{\ddagger}$ (kcal mol^{-1})	$\Delta S^{\ddagger}$ (cal K^{-1} mol^{-1})
k_{H^+}	26.4 ± 1.5	12.6 ± 1.0	29.7 ± 1.5	−2.9 ± 0.3
k_o	23.6 ± 3.5	7.4 ± 2.1	22.9 ± 3.5	−26.9 ± 13.2

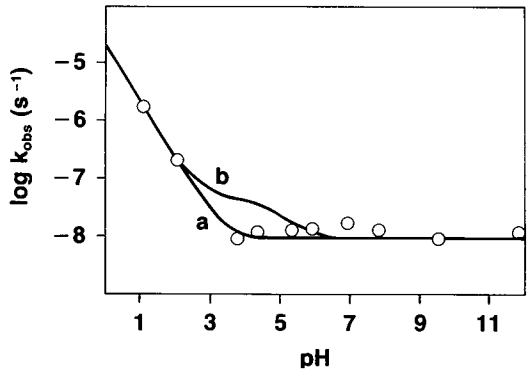


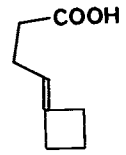
Fig. 2. Calculated pH–rate profile for the reaction of 5 at 60°C using $k_{H^+} = 2.25 \times 10^{-5}\ M^{-1}\ sec^{-1}$, $k_o = 9.23 \times 10^{-9}\ sec^{-1}$, $k_a = 1.58 \times 10^{-5}$, and (a) $k'_{H^+} = 0$ and (b) $k'_{H^+} = 2.25 \times 10^{-3}\ M^{-1}\ sec^{-1}$. The rate constants k_{H^+} and k'_{H^+} denote specific acid catalysis in the carboxyl and carboxylate forms of 5, respectively. The experimental data were obtained at 60°C and are shown here to indicate the lack of significant intramolecular acid catalysis.

intramolecular acid catalysis occurs [or the kinetically indistinquishable reaction of electrostatic catalysis by carboxylate (10)], then an inflection point in the pH–rate profile should be observed providing the catalysis is significant. This is shown in Fig. 2 using a value of $k'_{H^+} = 100k_{H^+}$, the same rate enhancement observed for prostacyclin. Comparison of the overlayed experimental data with the calculated curves shows that, if some intramolecular catalysis occurs, then its rate contribution must be much less than for prostacyclin. This same behavior (a lack of inflection about pH 4) is also observed in the higher-temperature profiles. Inasmuch as 5 does not show general acid catalysis in pH 4.6 acetate buffers at 80°C, it is not surprising that 5 lacks significant intramolecular catalysis. This conclusion is supported by the low EMs for intramolecular general catalysis observed elsewhere (24) and by the known insensitivity of general acid catalysis upon alkene hydration (18).

Reactivity of Prostacyclin Analogue 5. Most 1,1-dialkyl and trialkyl substituted alkenes show similar rates of hydration, ranging from 2 to 13 $\times\ 10^{-4} M^{-1} sec^{-1}$ at 25°C (Table IV). This insensitivity to alkyl-group substitution has been attributed to similar tertiary carbonium ion stabilization for the 1,1-dialkyl and trialkyl substituents. Inspection of Table IV shows that prostacyclin analogue 5 reacts almost 10^3 times slower than structurally similar trialkyl alkenes at 25°C. This rate retardation cannot be explained by a change in the rate-determining step from proton transfer ($k_{-1} < k_2 + k_3$, as occurs in most alkene hydrations) to preequilibrium proton transfer ($k_{-1} > k_2 + k_3$), as shown by the following isotope incorporation experiment.

When the reaction of 5 in deuterated hydrochloric acid was followed for approximately one half-life, no deuterium was incorporated into the remaining starting material. In these experiments, only the lactone products (7) contained deuterium (1 atom D = 100%). Preequilibrium proton transfer would necessitate isotope exchange in the remaining starting material, whereas rate-determining proton transfer would preclude it. The small solvent isotope effect observed for 5 is near unity ($k_{H^+}/k_{D^+} = 0.8 \pm 0.3$ at 60°C) and is not contradictory to an endothermic but rate-controlling proton transfer reaction. Most alkene hydration reactions show slightly positive k_{H^+}/k_{D^+} solvent isotope effects ranging from unity to ~3; their values are small because they are a composite of larger primary effects and inverse secondary effects. Inasmuch as the reaction of 5 is slower than for other trialkyl alkenes and therefore has a more product-like transition state, the primary isotope effect for 5 may be lower than for its analogues. Herein, the inverse secondary effect fully attenuates the primary isotope effect, causing an observed inverse k_{H^+}/k_{D^+} solvent isotope effect (Table IV).

The slow rate of hydration of 5, as compared to other alkenes, is partially due to the fused six-membered ring. This was demonstrated by measuring the rate of hydration of model compound 9 (Scheme V). Under similar reaction conditions, 9 gave $k_{H^+} = 9.3 \times 10^{-6}\ M^{-1}\ sec^{-1}$ at 25°C, somewhat smaller than for the other structurally similar alkenes in Table IV but still ~50 times faster than for 5. Thus,



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Scheme V

it is likely that the slow rate of 5 may be due both to the neighboring carboxyl group and to ring strain induced by the presence of neighboring cyclohexyl ring system. It has been reported previously that remote electron withdrawing groups strongly destabilize carbonium ion formation, as in the addition of trifluoroacetic acid to 5-substituted 1-hexenes (31,32). Further experiments to study this unusual type of stabilization are currently in progress.

Conclusions. Prostacyclin analogue 5 does not show significant intramolecular acid catalysis and, in fact, undergoes specific acid-catalyzed hydration approximately 10^3 times slower than expected as based on the reactivity of

Table IV. Specific Acid-Catalyzed Hydration Rate Constants and Solvent Isotope Effects for Various Olefins at 25°C

Alkene	Solution	$10^4\ k_{H^+}$ ($M^{-1}\ sec^{-1}$)	$\frac{k_{H^+}}{k_{D^+}}$	Ref. No.
5	HCl	0.0019	0.8[a]	—[b]
9	HCl	0.093		—[b,c]
Me_2C = CHMe	H_2SO_4	2.1	1.22	25, 26
Me_2C = CH_2	H_2SO_4	3.7	1.45	25, 27
Methylenecyclobutane	HNO_3	2.9		28, 29
1-Methylcyclopentene	H_2SO_4	9.1	0.93, 1.23	26, 29
1-Methylcyclohexene	$HClO_4$	6.2	1.16	29, 30
Methylenecyclohexane	$HClO_4$	13.4		30

[a] 60°C.

[b] This paper. Calculated from the Arrhenius parameters. The rate differences are not due to the use of HCl instead of H_2SO_4 or $HClO_4$; footnote *d* to Table I shows that the acid type does not affect the rate significantly.

[c] $E_a = 24 \pm 1$ kcal mol^{-1}.

other alkenes. The lack of isotope incorporation in the remaining reactant shows that proton transfer is rate determining. Prostacyclin analogue 5 is relatively stable in aqueous solution, even at low pH's partially because of stability conferred by the novel [4.2.0] ring system.

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