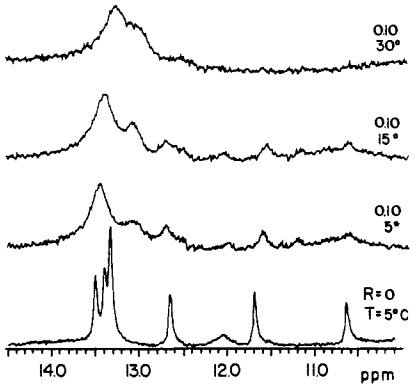

Figure 11. Effect of TMpyP(4) on the imino ^{1}H signals of d(TATATGTGCATATA)$_2$ (VI). The two imino signals of GT(7) are the two sharp signals to highest field. The similarity of the signals for AT(2)→GC(6) to that of d(TATATGCGCATATA)$_2$ (I) at $R = 0$ demonstrates that I and VI form similar duplexes, except for the central bp (7).

neighbor, bp, the upfield shift of the GC(6) NH signal might be explained since non-H-bonded G species have N(1)H signals at ~11 ppm.[24] However, we feel this explanation of the GC(6) NH shift in I-TMpyP(4) has several problems. First, GN(1)H exchanges very readily with H_2O.[24] However, with increasing temperature, the GC(6) NH signal of I-TMpyP(4) diminishes in intensity somewhat less than the GC(6) NH signal in I. Second, the TN(3)H signal of ĂC↓G in II-TMpyP(4) or T̆C↓G in III-TMpyP(4) is expected to be at ca. the same shift as the GN(1)H signal of C↓GC̆ in these adducts if base pairing is disrupted, since these signals for monomers are readily observed and have roughly the same chemical shift.[24,25] Third, the most plausible reason for not observing GC(6) NH exchange with H_2O for I-TMpyP(4) by this alternative model would be that the NH group is protected in a duplex. In such a case, we would have expected base stacking to shift the NH signals to higher field than 11 ppm[26] —not downfield at ~11.5 ppm as observed. Thus, at this juncture, our data suggest a binding mode in which the next neighbor bp are still hydrogen bonded. Furthermore, the observation of the GC(6) NH signal in I-TMpyP(4) and the ST experiment depicted in Figure 8 require that the interconversion of I and I-TMpyP(4) proceeds without appreciable NH-H_2O exchange.

The specific details of the interaction of the TMpyP(4) with the near-neighbor bp and the evaluation of the retention of H bonding by these base pairs must await the discovery of an even more selective interaction. However, for a species that adds to DNA noncovalently, the synthetic TMpyP(4) cation exhibits unprecedented selectivity for 5′CG3′ over 5′GC3′, 5′GG3′, 5′TG3′, 5′GT3′, and 5′GA3′ sequences. The complex natural product, actinomycin D, is the only intercalator known to exhibit pronounced specificity for one site (5′GC3′).[17a,21,27] However, this anticancer antibiotic binds to other dinucleotide sequences containing G. Although this study provides the first direct evidence consistent with intercalation of TMpyP(4) at "GC" but not at "AT" sites, more studies are needed to further define the adducts formed by DNA binding porphyrins.

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Registry No. 2I·TMpyP(4), 102573-50-2; 2II·TMpyP(4), 102573-51-3; 2III·TMpyP(4), 102573-52-4; IV, 102505-17-9; V, 102520-98-9; VI, 102505-18-0; TMpyP(4), 38673-65-3; TMAP, 69458-19-1; TMpyP(4)·2poly[d(G-C], 102535-16-0.

(24) In unpublished studies, we find that cGMP(2′,3′) has an N(1)H signal at 11.0 ppm in H_2O at pH 6.4-4.0. Under similar conditions, the spectrum of TMP has an N(3)H signal at 11.1 ppm for pH 4.9-2.5.
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Prostacyclin: Evidence That Intramolecular General-Acid Catalysis by Its Carboxylic Acid Group Is Responsible for the Extra Hydrolytic Lability

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Abstract: Rates of hydrolysis of the vinyl ether functional group of prostacyclin and its methyl ester were measured in aqueous solution at 25 °C over the acidity range -log [H$^+$] = 1-8. The rate profile for prostacyclin shows a break, from which pK_a = 5.03 ± 0.15 may be inferred for the carboxylic acid group of this molecule and a 10^2-fold greater reactivity may be deduced for the carboxylate over the carboxylic acid form. The hydrogen ion catalytic coefficient for reaction of the carboxylic acid form, k_{H^+} = 439 ± 4 M^{-1} s^{-1}, is similar to that of prostacyclin methyl ester, k_{H^+} = 418 ± 5 M^{-1} s^{-1}, and is normal for a vinyl ether of this structure. Kinetic isotope effects and unusually weak catalysis by external general acids suggest that the abnormal reactivity of the carboxylate form is the result of intramolecular general-acid catalysis by the carboxylic acid group; an effective molarity of 0.6 M can be estimated for this process.

Prostacyclin (**1**, R = H; Scheme I) is a recently discovered[1] eicosanoid with remarkable anti-blood-clotting properties.[2] This gives it great potential as a therapeutic agent for the treatment of thrombosis and also as an anticlotting factor to confer non-

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[‡] Upjohn Co.

(1) Moncada, S.; Gryglewski, R.; Bunting, S.; Vane, J. R. *Nature (London)* **1976**, *263*, 663-665.

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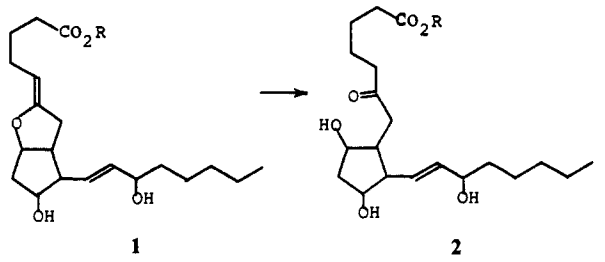
Scheme I



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coagulant properties upon vascular prosthetic devices. Prostacyclin's usefulness, however, is drastically limited by instability: its lifetime under physiological conditions is only 3 min.

It was shown early in the short history of prostacyclin that this instability converts the substance into the ketone **2**[3] and that the decomposition reaction is therefore very probably acid-catalyzed hydrolysis of the vinyl ether group of the prostacyclin molecule. This was quickly confirmed by a kinetic study,[4] but that investigation also revealed that this vinyl ether group is unusually labile. Our further examination showed the hydronium ion catalyzed hydrolysis to be accelerated by prostacyclin's carboxylic acid group operating from the carboxylate form.[5]

We pointed out in the latter study that this extra lability could be due either to an electrostatic effect of the carboxylate group or to intramolecular general-acid catalysis. Different isotope effects can be predicted for these two mechanistic explanations. We have now performed the necessary isotopic measurements and find that the results favor intramolecular general-acid catalysis. We report that work here, and we also describe more fully our previous study, which was originally communicated only in preliminary form.[5]

Accelerations of the rate of vinyl ether hydrolysis attributable to the action of a nearby carboxylate group have been observed before in several other systems, and the effects have been discussed in terms of nucleophilic participation[6] as well as electrostatic facilitation[6,7] and intramolecular general-acid catalysis.[7]

Experimental Section

Materials. Prostacyclin sodium salt and prostacyclin methyl ester were gifts supplied by Drs. U. F. Axen and J. E. Pike of Upjohn Co. Methylphosphonic acid was prepared from dimethyl methylphosphonate;[8] all other materials were best available commercial grades. Solutions were made from deionized H_2O, purified further by distillation, or from D_2O (Merck Sharp and Dohme; 99.8 atom % D) as received.

Kinetics. Rates of hydrolysis were measured spectroscopically by monitoring the decrease of the strong absorbance of the vinyl ether group of prostacyclin and prostacyclin methyl ester at 200–210 nm. Measurements were made either with a Cary Model 118 spectrometer whose cell compartment was thermostated at 25.0 ± 0.02 °C or with Durrum Gibson or Nortech stopped-flow spectrometers operating at 25.0 ± 0.1 °C. Initial substrate concentrations were ca. 10^{-4} M, and ionic strength was generally maintained (using NaCl) at 0.10 M, except in carboxylic acid buffers, where it was 0.040 M. Slow runs measured with the Cary spectrometer were performed by first allowing 3 mL of buffer solution contained in a cuvette to come to temperature equilibrium with the cell compartment and then adding 3 μL of methanol stock solution of substrate. For the fast runs measured with the stopped-flow spectrometers, aqueous substrate and acid or buffer solutions were mixed in a 1:1 ratio. In order to slow unwanted hydrolysis in the methanol stock and aqueous substrate solutions of prostacyclin, these solutions were made weakly alkaline (4 × 10^{-4} M NaOH). The kinetic data conformed to the first-order rate law well, and observed rate constants were evaluated by analyzing the relationship between ln $(A - A_\infty)$ and time.

Table I. Rate Constants for the Hydrolysis of Prostacyclin Methyl Ester in Aqueous Solution at 25 °C

catalyst	pK_a	k/M^{-1} s^{-1}
H_3O^+	−1.74	418
HCO_2H	3.75	3.21
$HOCH_2CO_2H$	3.83	3.81
CH_3CO_2H	4.76	0.925
$C_2H_5CO_2H$	4.88	0.922
$CH_2ClPO_3H^-$	6.59	0.195
$CH_3PO_3H^-$	8.00	0.0175
$NH_3^+(CH_2)_2NH_3^+$	6.85	0.000883
D_3O^+ [a]	−1.74	140
CH_3CO_2D [a]	5.27	0.184

[a] In D_2O.

Table II. Rate Constants for the Hydrolysis of Prostacyclin in Aqueous Solution at 25 °C

catalyst	k^a/M^{-1} s^{-1}	k'^b/M^{-1} s^{-1}
H_3O^+	439	43600
D_3O^+ [c]		32900
$H_2PO_4^-$		0.048
$D_2PO_4^-$ [c]		0.0068

[a] Rate constant for reaction of the carboxylic acid form. [b] Rate constant for reaction of the carboxylate form. [c] In D_2O.

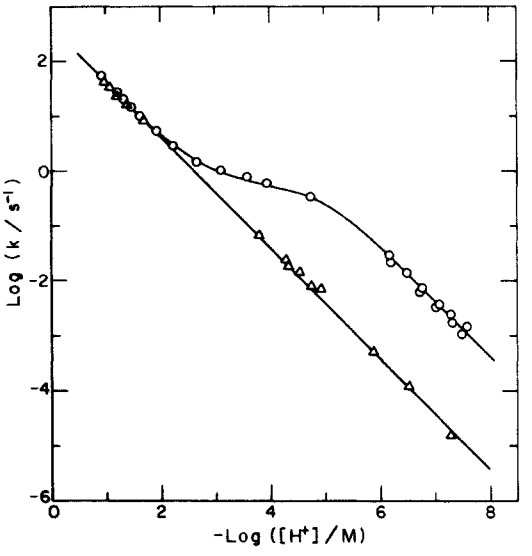

Figure 1. Rate profile for the hydrolysis of the vinyl ether functional group of prostacyclin (○) and prostacyclin methyl ester (△) in aqueous solution at 25 °C.

Results

Prostacyclin Methyl Ester. Rates of reaction of this substance were measured in dilute perchloric acid and various buffer solutions in H_2O and in dilute hydrochloric acid and acetic acid buffers in D_2O. The data are summarized in Tables S1–S4.[9]

All of the reactions monitored were many orders of magnitude faster than known rates of hydrolysis of simple carboxylic acid esters,[10] and the ester functional group therefore remained intact under the present investigative conditions.

The vinyl ether hydrolysis reaction observed was strongly catalyzed by undissociated acids in addition to the hydronium ion;

(2) For recent reviews of the physiological and chemical properties of prostacyclin, see: Bartmann, W.; Beck, G. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 751–764. Nelson, N. A.; Kelly, R. C.; Johnson, R. A. *Chem. Eng. News* **1982**, *60*(30), 30–44.

(3) Johnson, R. A.; Morton, D. R.; Kinner, J. H.; Gorman, R. R.; McGuire, J. C.; Sun, F. F.; Whittaker, N.; Bunting, S.; Salmon, J.; Moncada, S.; Vane, J. R. *Prostaglandins* **1976**, *12*, 915–928. Corey, E. J.; Keck, G. E.; Szekely, I. *J. Am. Chem. Soc.*, **1977**, *99*, 2006–2008.

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(5) Chiang, Y.; Kresge, A. J.; Cho, M. J. *J. Chem. Soc., Chem. Commun.*, **1979**, 129–130.

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(7) Loudon, G. M.; Smith, C. K.; Zimmerman, S. E. *J. Am. Chem. Soc.* **1974**, *96*, 465–479. Loudon, G. M.; Ryono, D. E. *J. Am. Chem. Soc.* **1976**, *98*, 1900–1907.

(8) Kluger, R. H.; Wasserstein, P.; Nakoda, K. *J. Am. Chem. Soc.* **1975**, *97*, 4298–4303.

(9) Supplementary material. See paragraph at the end of this paper.

(10) *Natl. Bur. Stand. Circ. (U.S.)* **1951**, *No. 510*; **1956**, *Suppl. 1*, Table 212.441.

it showed no catalysis by bases. The rate data were therefore fitted to eq 1: linear least-squares analysis of the relationship between

$$k_{obsd} = k_{H^+}[H^+] + k_{HA}[HA] \quad (1)$$

k_{obsd} and [HA] for series of measurements made at constant buffer ratio gave general-acid catalytic coefficients, k_{HA}, as slopes and contributions to reaction by the hydronium ion, $k_{H^+}[H^+]$, as intercepts. Values of k_{HA} so obtained are listed in Table I, and the intercepts, together with observed rate constants measured in perchloric acid solution, are displayed in the rate profile of Figure 1. Linear least-squares analysis of the relationship between log k and log $[H^+]$[11] gave a slope of unity, 0.998 ± 0.011, which shows that the reaction is exactly first order in hydronium ion over the change in $[H^+]$ of 6 orders of magnitude for which measurements were made. A best value of k_{H^+} was obtained by weighted[13] least-squares analysis of the dependence of all values of k upon $[H^+]$; this gave $k_{H^+} = 418 \pm 5$ M^{-1} s^{-1}[14] and an "uncatalyzed" or solvent-mediated reaction rate constant indistinguishable from zero, $(0.06 \pm 4.31) \times 10^{-4}$ s^{-1}.

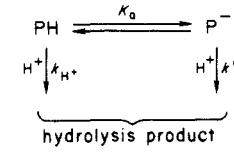
The catalytic coefficients for the four carboxylic acids used in this study give a Brønsted relation whose exponent, $\alpha = 0.58 \pm 0.05$, is not unlike those observed for the hydrolysis of other vinyl ethers.[15] The catalytic coefficients for the two phosphonate anions lie above this Brønsted correlation line, and those for the hydronium ion and diprotonated ethylene diamine lie below it; this again is normal behavior.[15]

The rate measurements using acetic acid buffers in D_2O solution gave a hydronium ion catalytic coefficient, $k_{D^+} = 149 \pm 5$ M^{-1} s^{-1},[16] that is consistent with the value measured directly in D_2O solutions of hydrochloric acid, $k_{D^+} = 133 \pm 5$ M^{-1} s^{-1}. The result listed in Table I is the weighted average of these two values; it gives the hydronium ion isotope effect, $k_{H^+}/k_{D^+} = 2.99 \pm 0.08$, and the ratio of the two acetic acid catalytic coefficients gives the general acid isotope effect, $k_{HOAc}/k_{DOAc} = 5.02 \pm 0.15$. These isotope effects are again normal values for this kind of reaction.[15a,18]

Prostacyclin. Rates of hydrolysis of the vinyl ether group of prostacyclin were measured in dilute hydrochloric acid and several buffer solutions in H_2O and in biphosphate buffer solutions in D_2O. The data are summarized in Tables S5–S7.[9]

Reaction of this substrate, unlike that of the ester, was catalyzed only weakly, if at all, by general acids, especially in buffer solutions of low $[H^+]$. Reliable values of general-acid catalytic coefficients could therefore not be obtained in most cases. On the other hand, because the slopes of plots of k_{obsd} vs. buffer acid concentration were so shallow, hydronium ion contributions, $k_{H^+}[H^+]$, could be determined with good accuracy. These, together with observed rate constants measured in hydrochloric acid solutions, are displayed in Figure 1.

It may be seen that the dependence of reaction rate on $[H^+]$ is complex, with separate linear relationships at low and at high pH. The transition from one linear portion to the other occurs in a region where ionization of the carboxyl group of prostacyclin can be expected to occur, and this suggests that conversion of this group from the acid to the basic form changes the reactivity of the vinyl ether function. The data were therefore fitted to a reaction scheme based upon this hypothesis. This is shown in eq 2, where PH represents prostacyclin in the un-ionized carboxylic



(2)

acid form and P^-, in the ionized carboxylate form. The rate law relating observed first-order hydronium ion rate constants to the species-specific rate constants of this scheme is given in eq 3.

$$(k_{obsd})_{H^+} = \frac{k_{H^+}[H^+]^2 + k_{H^+}'K_a[H^+]}{[H^+] + K_a} \quad (3)$$

Weighted[13] least-squares fitting of the data to this expression produced $K_a = (1.29 \pm 0.46) \times 10^{-5}$ M,[14] $k_{H^+} = (4.39 \pm 0.04) \times 10^2$ M^{-1} s^{-1},[14] and $k_{H^+}' = (4.43 \pm 1.42) \times 10^4$ M^{-1} s^{-1}.[14]

Rate measurements in the region of the rate profile that determines K_A were done at the ionic strength $\mu = 0.040$ M, and this equilibrium constant may therefore be adjusted to its zero ionic strength value by using activity coefficients appropriate to $\mu = 0.040$ M.[12] This gives $pK_a = 5.03 \pm 0.15$, which is a reasonable value for a carboxylic acid group such as that in prostacyclin, e.g., $pK_a = 4.82$–4.88 for propanoic to hexanoic acids.[19] This agreement with expectation gives support to the reaction scheme of eq 2.

This acidity constant is 2 orders of magnitude greater than the hydrogen ion concentrations of the biphosphate buffers used to measure rates of hydrolysis of prostacyclin in D_2O solution, and these experiments were consequently performed under conditions where the carboxyl group was essentially completely ionized. The results therefore provide a value of the hydronium ion catalytic coefficient for hydrolysis of the carboxylate form of prostacyclin in D_2O: $k_{D^+}' = (3.29 \pm 0.10) \times 10^4$ M^{-1} s^{-1}.[20] This can be combined with $k_{H}' = (4.36 \pm 0.09) \times 10^4$ M^{-1} s^{-1}, obtained from analogous experiments in biphosphate buffers in H_2O (which give a more accurate value of k_{H^+}' than that derived from the rate profile), to give the isotope effect $k_{H^+}'/k_{D^+}' = 1.33 \pm 0.05$. This value is rather low for straightforward hydronium ion catalyzed hydrolysis of a vinyl ether as reactive as prostacyclin; its mechanistic significance is discussed below.

General-acid catalysis in these biphosphate buffer solutions was weak, and these experiments did not define general-acid catalytic coefficients at all well. Nevertheless, values may be obtained that do give at least an indication of the magnitude of the isotope effect on the general-acid-catalyzed reaction. Analysis of these kinetic data must take into account general-acid reactions of both carboxyl and carboxylate forms of the substrate, as in eq 2 for hydronium ion catalysis. In addition, reaction could occur through undissociated phosphoric acid as well as through biphosphate ion: though the concentration of the former in these buffer solutions is very much less than that of the latter, it is a much stronger acid and will have a correspondingly greater rate constant; catalysis by phosphoric acid in biphosphate buffers has in fact been detected in the hydrolysis of some vinyl ethers[22] and in other general-

(11) Values of $[H^+]$ for the buffer solutions were obtained by calculation using literature pK_a's of the buffer acids and activity coefficients recommended by Bates.[12]

(12) Bates, R. G. *Determination of pH. Theory and Practice*; Wiley: New York, 1973; p 49.

(13) Weights were assigned in inverse proportion to values of the rate constants. This was done because the methods of measurement produced uncertainties in rate constants that decreased as the rate constants decreased; i.e., absolute uncertainties were not constant, but relative uncertainties were approximately so.

(14) This value is slightly different from the result we published in preliminary form[5] because we have added to and refined our original data.

(15) (a) Kreevoy, M. M.; Eliason, R. *J. Phys. Chem.* **1968**, *72*, 1313–1316. (b) Lienhard, G. E.; Wang, T.-C. *J. Am. Chem. Soc.* **1969**, *91*, 1146–1153. Kresge, A. J.; Chen, H. L.; Chiang, Y.; Murrill, E.; Payne, M. A.; Sagatys, D. S. *J. Am. Chem. Soc.* **1971**, *93*, 413–423. Kresge, A. J.; Chen, H. L. *J. Am. Chem. Soc.* **1972**, *94*, 2818–2822. (c) Kresge, A. J.; Chiang, Y. *J. Am. Chem. Soc.* **1973**, *95*, 803–806. Chwang, W. K.; Eliason, R.; Kresge, A. J. *J. Am. Chem. Soc.* **1977**, *99*, 805–808. (d) Kresge, A. J.; Chwang, W. K. *J. Am. Chem. Soc.* **1978**, *100*, 1249–1253. Chiang, Y.; Chwang, W. K.; Kresge, A. J.; Robinson, L. H.; Sagatys, D. S.; Young, C. I. *Can. J. Chem.* **1978**, *56*, 456–459.

(16) Values of $[D^+]$ needed to analyze these data were calculated by using a solvent isotope effect on the ionization of acetic acid of $\Delta pK_a = 0.514$.[17]

(17) (a) Gary, R.; Bates, R. G.; Robinson, R. A. *J. Phys. Chem.* **1965**, *69*, 2750–2753. (b) Gold, V.; Lowe, B. M. *J. Chem. Soc. A* **1968**, 1923–1932.

(18) (a) Salomaa, P.; Kankaanpera, A.; Lajunen, M. *Acta Chem. Scand.* **1966**, *20*, 1790–1801. Kresge, A. J.; Chiang, Y. *J. Chem. Soc. B* **1967**, 58–61. (b) Kresge, A. J.; Sagatys, D. S.; Chen, H. L. *J. Am. Chem. Soc.* **1977**, *99*, 7228–7233.

(19) Bell, R. P. *The Proton in Chemistry*; Cornell University: Ithaca, NY, 1973; p 75.

(20) Values of $[D^+]$ needed for this analysis were calculated by using a solvent isotope effect on the ionization of biphosphate ion of $\Delta pK_a = 0.535$.[21]

(21) Gary, R.; Bates, R. G.; Robinson, R. A. *J. Phys. Chem.* **1964**, *68*, 3806–3809.

acid-catalyzed reactions.[23] These considerations lead to the rate law of eq 4 for that part of the reaction catalyzed by undissociated

$$\frac{\Delta k_{obsd}}{\Delta[H_2PO_4^-]} = [(k_{H_3PO_4}/K_1)[H^+]^2 + [k_{H_2PO_4} + k'_{H_3PO_4}K_a/K_1][H^+] + k'_{H_2PO_4}K_a]/([H^+] + K_a) \quad (4)$$

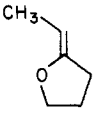
acids; in this expression unprimed rate constants represent reaction of prostacyclin in its carboxylic acid form and primed constants, in the carboxylate form, K_1 is the first ionization constant of phosphoric acid, and K_a is the acidity constant of prostacyclin. In the buffers used, $[H^+]$ was very much less than K_a, and this rate law then reduces to that shown in eq 5.

$$\frac{\Delta k_{obsd}}{\Delta[H_2PO_4^-]} = \frac{k_{H_3PO_4}}{K_1K_a}[H^+]^2 + \left[\frac{k_{H_2PO_4}}{K_a} + \frac{k'_{H_3PO_4}}{K_1}\right][H^+] + k'_{H_2PO_4} \quad (5)$$

The data for both H_2O and D_2O solutions do show increasing catalysis by buffer acid species with increasing hydronium ion concentration, but no curvature in the relationship between these two variables could be detected. Apparently, therefore, the quadratic term of eq 5 makes no contribution under the conditions employed. The data were consequently analyzed by linear least-squares methods, and the following results were obtained: for H_2O solution, $\Delta k_{obsd}/\Delta[H_2PO_4^-] = (4.79 \pm 0.85) \times 10^{-2} + (1.04 \pm 0.19) \times 10^5[H^+]$; for D_2O solution, $\Delta k_{obsd}/\Delta[D_2PO_4^-] = (6.82 \pm 1.33) \times 10^{-3} + (4.26 \pm 2.05) \times 10^4[D^+]$. The intercepts of these relationships give the isotope effect $k'_{H_2PO_4}/k'_{D_2PO_4} = 7.0 \pm 1.8$, which, though not very precise, is a reasonable value for the hydrolysis of a vinyl ether catalyzed by a general acid. The slopes, of course, do not represent single rate or equilibrium constants; nevertheless, the isotopic ratio that they give, 2.4 ± 1.3, is also reasonable: each slope is the sum of two rate constants each divided by an acid dissociation constant, and the individual isotope effects on the rate constants could well be twice as great as those on the acid dissociation constants.

Discussion

The hydronium ion catalytic coefficient determined here for hydrolysis of the vinyl ether functional group of prostacyclin methyl ester, $k_{H^+} = 418\ M^{-1}\ s^{-1}$, is similar to that measured for prostacyclin in its un-ionized carboxylic acid form, $k_{H^+} = 439\ M^{-1}\ s^{-1}$. Both values, moreover, are not unlike hydronium ion catalytic constants for the hydrolysis of simple monofunctional vinyl ethers of similar structure, for example, $k_{H^+} = 635\ M^{-1}\ s^{-1}$ for the hydrolysis of **3** and $k_{H^+} = 314\ M^{-1}\ s^{-1}$ for the hydrolysis of **4**.[24]



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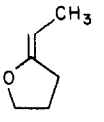

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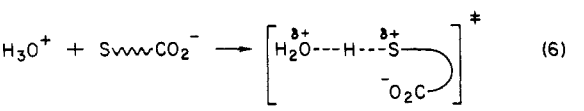
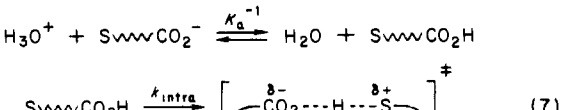
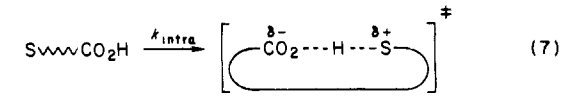
The vinyl ether groups of un-ionized prostacyclin and prostacyclin methyl ester therefore appear to have normal reactivity.

The hydronium ion catalytic coefficient for hydrolysis of the vinyl ether group of prostacyclin in the carboxylate form, on the other hand, $k_{H^+} = 43\,600\ M^{-1}\ s^{-1}$, is abnormally high; it is 104 times the corresponding rate constant for prostacyclin methyl ester and 99 times that for un-ionized prostacyclin. We have speculated before that this rate acceleration could be either due to the result of a stabilizing electrostatic interaction between the carboxylate group and the positive charge being generated on the substrate in the transition state of this reaction, as illustrated schematically in eq 6, or due to protonation of the carboxylate group followed by intramolecular general-acid catalysis, as shown in eq 7. These



(6)





(7)

two mechanisms should give different solvent isotope effects, inasmuch as the nonreacting O–H bonds of the former hydronium ion still bear some positive charge in the transition state of eq 6 whereas they have been completely converted to uncharged O–H bonds by the time the transition state is reached in eq 7. The mechanisms might therefore be distinguished on this basis.

A quantitative prediction of the solvent isotope effect on the scheme of eq 6 can be made from a relationship we have established between hydronium ion isotope effects for the hydrolysis of a group of some 30 vinyl ethers and the free energies of activation of these reactions.[18b] This correlation gives $k_{H^+}/k_{D^+} = 3.6$ for a free energy of activation corresponding to the present rate constant, $k_{H^+}' = 41\,300\ M^{-1}\ s^{-1}$. The mechanism of eq 7 requires a somewhat different method for making a quantitative estimate, since here the active proton donor is not a hydronium ion, and in this case it is useful to employ the method of fractionation factors.[25] This method expresses an isotope effect as the product of fractionation factors for all exchangeable hydrogens of the initial state divided by a like product for the transition state. Application of this formula to the present system gives eq 8 in

$$k_{H^+}/k_{D^+} = l^3\Phi/\phi^* \quad (8)$$

which l is the fractionation factor for the hydronium ion, ϕ^* is that for the hydrogen being transferred in the transition state, and Φ accounts for isotopic fractionation in the solvation shell of the carboxylate ion. The value of l (=0.69) is well-known,[25] $\Phi = 0.90$ has been determined for the acetate ion,[17b,25b] and for ϕ^* we may use a value based on the presently measured isotope effect for hydrolysis of prostacyclin methyl ester catalyzed by acetic acid: $\phi^* = \phi_{LOAc}(k_{HOAc}/k_{DOAc})^{-1} = 0.19$ ($\phi_{LOAc} = 0.96$[17b] is the fractionation factor for the acidic hydrogen of acetic acid). This leads to the prediction $k_{H^+}/k_{D^+} = 1.5$.

The second of these predictions is in much better agreement with the value of the isotope effect on this reaction measured here, $k_{H^+}'/k_{D^+}' = 1.33 \pm 0.05$. This suggests that the mechanism of eq 7 involving intramolecular general-acid catalysis provides a better explanation of the extra hydrolytic reactivity of the vinyl ether group of ionized prostacyclin than does the electrostatic stabilization illustrated in eq 6.

This mechanistic conclusion is supported by the very weak, or even sometimes nonexistent, general-acid catalysis found here for the hydrolysis of the vinyl ether group of prostacyclin in the carboxylate form. At low $[H^+]$, hydrolysis occurs virtually completely by reaction through the hydronium ion; catalysis by undissociated acids makes up very little if any of the total reaction rate. This is unusual behavior, for general-acid catalysis ordinarily becomes more prominent as $[H^+]$ is lowered; it follows, for example, from the Brønsted relation that the hydronium ion contribution to observed rates, $k_{H^+}[H^+]$, should diminish in importance relative to the general-acid contribution, $k_{HA}[HA]$, as $[H^+]$ is lowered and weaker general acids are used,[26] and such a pattern is in fact shown by the results obtained here for hydrolysis of the vinyl ether group in prostacyclin methyl ester. The dominance

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of the hydronium ion contribution found for prostacyclin at low $[H^+]$ must mean that the accelerative effect of the carboxylate group operates chiefly only in the hydronium ion reaction: there is little or no corresponding acceleration of general-acid-catalyzed pathways. This would seem unlikely if the carboxylate group were acting electrostatically, for that group should exert its effect in transition states formed from general acids as well as those from the hydronium ion. An electrostatic acceleration, if present, should in fact be especially prominent in reactions of positively charged general acids such as the *N*-methylmorpholinium ion studied here, but general-acid catalysis by this species was found to be barely detectable. Electrostatic effects, moreover, on proton-transfer reactions in aqueous solution are ordinarily quite small; they are of a magnitude approaching the present rate acceleration only when the proton is completely transferred and the charge is completely developed at the rate-determining transition state,[27] and in cases of partial proton transfer such as vinyl ether hydrolysis they are an order of magnitude smaller.[15c]

It has been suggested by a reviewer that the rate acceleration shown by prostacyclin in its carboxylate form may have effected a change in mechanism of this reaction from the conventional scheme for vinyl ether hydrolysis, in which carbon protonation is rate determining, to one where this step is reversible and subsequent reaction of the alkoxycarbocation intermediate is rate determining. We believe that this has not happened, for such a preequilibrium process should give an inverse hydronium ion isotope effect, $k_{H^+}/k_{D^+} < 1$, as has indeed been reported for the single example of such a mechanism for vinyl ether hydrolysis found to date.[28] We are nevertheless exploring this mechanistic possibility and will report our findings separately.

Rate accelerations attributable to intramolecular catalysis are commonly expressed as effective molarities. An effective molarity can be estimated for the present case by comparing the rate constant for the intramolecular step of the present reaction, $k_{intra} = k_{H^+}'K_a = 0.53\ s^{-1}$ (cf. eq 7), with the specific rate of hydrolysis of prostacyclin in the carboxylic acid form catalyzed by an external carboxylic acid of the same pK_a. Such a value is not available, but the similarity of the rates of hydrolysis of prostacyclin and its methyl ester noted above and evident in Figure 1 suggests that the ester may be a good model, and the rate constant for the hydrolysis of that substance by acetic acid is available: $k_{HOAc} = 0.93\ M^{-1}\ s^{-1}$. Use of this result leads to an effective molarity of 0.57 M for the present intramolecular reaction. This is a rather small value, but effective molarities for general-acid- and general-base-catalyzed reactions do tend to be small, with the important exception of the hydrolysis of certain acetals.[29]

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Registry No. D_2, 7782-39-0; H_3O^+, 13968-08-6; HCO_2H, 64-18-6; $HOCH_2CO_2H$, 79-14-1; CH_3CO_2H, 64-19-7; $C_2H_5CO_2H$, 79-09-4; $CH_2ClPO_3H^-$, 54947-16-9; $CH_3PO_3H^-$, 39863-50-8; $NH_3^+(CH_2)_2NH_3^+$, 22534-20-9; D_3O^+, 24847-51-6; CH_3CO_2D, 758-12-3; $H_2PO_4^-$, 14066-20-7; $D_2PO_4^-$, 69976-02-9; prostacyclin, 35121-78-9; prostacyclin methyl ester, 61799-74-4.

Supplementary Material Available: Tables of rate data (15 pages). Ordering information is given on any current masthead page.

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Hydrogen Bonding between Solutes in Aqueous Solution[1]

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Contribution No. 1587 from the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254. *Received November 13, 1985*

Abstract: Hydrogen bonding between protonated amines and substituted phenolate ions in water has been measured from the increase in phenolate absorbance with increasing concentration of amine buffers, at pH values below the pK_a of the phenol. This method permits measurement of hydrogen bonding between phenolate ion and acids of lower pK_a than phenol. Hydrogen bonding is weak, with an association constant of $K_{AB} = 0.81\ M^{-1}$ for the complex of phenolate ion and ethylenediamine dication at ionic strength 2.0 M (KCl), 25 °C. The absorption spectra of the hydrogen-bonded complexes are similar to those of the corresponding phenolate ions in water. This shows that the hydrogen-bonded proton in the complex is in a double-minimum energy well, with a significant barrier for transfer in the thermodynamically favorable direction. Hydrogen bonding to phenolate ion increases with increasing strength of the acid and follows a Brønsted correlation with $\alpha = 0.15$ for substituted ammonium cations. Values of K_{AB} for complexes with ethylenediamine dication increase with increasing basicity of substituted phenolate ions and are consistent with a Brønsted slope of $\beta = 0.10$. The results may be described by a Hine interaction coefficient of $\tau = \partial\alpha/\partial pK_{BH} = \partial\beta/-\partial pK_{AH} = 0.013$. Thiol anions and *p*-nitrophenolate anion exhibit weaker hydrogen-bonding ability.

The importance of hydrogen bonding for maintaining the structure of biological macromolecules and in general acid-base catalysis is well known, but remarkably little is known about the strength of hydrogen bonds between solutes in aqueous solution. The formation of such hydrogen bonds requires that competition from hydrogen bonding of the donor and acceptor to 55 M water must be overcome (eq 1), but it has not been generally appreciated

$$B{\cdot}HOH + H_2O{\cdot}HA \rightleftharpoons B{\cdot}HA + H_2O{\cdot}HOH \quad (1)$$

what is necessary in order to overcome this competition. It is clear that intermolecular hydrogen bonding in water is generally weak, with association constants that are often not significantly larger than that expected for the formation of random-encounter complexes.[2-5] An exception is the hydrogen bifluoride ion, FHF^-,

(1) This paper is dedicated to Jack Hine. The research was supported in part by grants from the National Institutes of Health (GM 20888) and the National Science Foundation (PCM 81-17816).
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