Table I—Inhibitory Effect of Adenosine and Fractions Extracted from *U. pertusa* on the Contractility of Isolated Guinea Pig Atria [a]

Substance	Concentration, g/ml	Inhibition,[b] %
Crude oil	4×10^{-4}	6.9
Fraction 9[c]	4×10^{-5}	40.4
Final crystals	1×10^{-5}	63.6
Adenosine	1×10^{-5}	69.0

[a] The guinea pig left atria was electrically driven with square pulses (2 Hz, 5 msec duration, and 5 V). [b] Inhibition was expressed by percent decrease in the systolic tension development of the atria. [c] Purified using column chromatography (TSK-Gel).

that of authentic adenosine, 1×10^{-5} g/ml. Figure 1 is representative of the negative inotropic effect of the purified crystalline material (1×10^{-5} and 3×10^{-5} g/ml) on the atria.

When the crystalline material was washed out of the tissue bath, the negative inotropic action of the agent disappeared. The contractility was restored to the control level within 1 min after washout, similar to the effect of the removal of adenosine reported by Baumann *et al.* (5). Furthermore, negative inotropic effects of the purified crystalline material and adenosine were not affected by the β-blocker propranolol (1×10^{-6} *M*) or the α-blocker phentolamine (1×10^{-6} *M*). Thus, the inhibitory action of the purified crystalline material is not mediated through the activation of adrenoceptors. In conclusion, the chemical and pharmacolgical results indicate that the cardioinhibitory substance isolated from the green alga *U. pertusa* is adenosine, which had not been found previously in marine organism.

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ACKNOWLEDGMENTS

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Degradation of Fenprostalene in Aqueous Solution

DAVID M. JOHNSON [x], WILLIAM F. TAYLOR, GEOFFREY F. THOMPSON, and RAYMOND A. PRITCHARD

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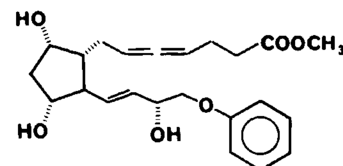
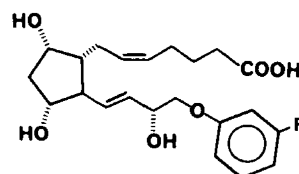
Abstract ☐ The degradation of the prostaglandin fenprostalene (III) was studied in aqueous solution. The reaction was both specific acid and base catalyzed. The only reaction found to occur was hydrolysis of the methyl ester at C-1. Activation energies for the acid- and base-catalyzed reactions were determined and are nearly identical to that for the hydrolysis of ethyl acetate, a model ester. A competing acid-catalyzed reaction of the C-1 free acid of III was found to be ~ 10 times slower than the hydrolysis of III.

Keyphrases ☐ Degradation—fenprostalene, prostaglandins, high-performance liquid chromatography, kinetics, ester hydrolysis ☐ Fenprostalene—degradation, prostaglandins, high-performance liquid chromatography, kinetics, ester hydrolysis ☐ High-performance liquid chromatography—degradation of fenprostalene, kinetics, ester hydrolysis ☐ Ester hydrolysis—fenprostalene degradation, high-performance liquid chromatography, kinetics



FENPROSTALENE (III)



I: $R = CF_3$
II: $R = Cl$

The degradation of prostaglandin $F_{2\alpha}$ in aqueous solution is known to be acid catalyzed (1, 2). No evidence of degradation under alkaline conditions has been reported. Recently, the degradation of two $F_{2\alpha}$-type aryloxy prostaglandins (I and II) was reported by Jones *et al.* (3). The authors found that the degradation of these luteolytic agents in aqueous solution was acid catalyzed, and the rate of degradation as well as the type of products formed were dependent on the presence of oxygen in the solution. No degradation of these compounds was observed at pH values >5.

Fenprostalene (III)[1] is a new prostaglandin developed for use as an abortifacient and for estrus synchronization in cattle (4, 5). The likely degradation route of this F-type prostaglandin at high pH is hydrolysis of the C-1 methyl ester group. However, we were interested to see whether acid-catalyzed decomposition reactions analogous to those found for I and II (3) or $PGF_{2\alpha}$ itself (1, 2) might compete with acid-catalyzed hydrolysis of the ester moiety in III at low pH. Accordingly, we have studied the degradation of fenprostalene (III) and its C-1 free acid (IV) in aqueous solution as a function of pH.

EXPERIMENTAL

Materials—The fenprostalene (6) used was 99% pure by high-performance liquid chromatographic (HPLC) area normalization. The methanol was glass-distilled HPLC grade, and the water was purified through a filtration and ion exchange system[2]. All other chemicals were reagent grade quality.

Kinetics Methods—Buffer solutions contained 0.025 *M* total buffer,

[1] Fenprostalene is the generic name for methyl 7-[3,5-dihydroxy-2-(3-hydroxy-4-phenoxy-1-butenyl)cyclopentyl]-4,5-heptadienoate.

[2] Barnstead Nanopure System.

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Table I—Observed Rate Constants for the Decomposition of III and IV in Aqueous Solution

Compound	pH	Buffer[a]	Temperature	$k_{obs} \times 10^7$ (sec^{-1})
III	1.15	HCl	80°	3360
	2.99[b]	Formate	80°	35.0
	3.21	Formate	80°	22.1
	3.22[c]	Formate	80°	21.6
	5.13	Acetate	80°	1.43
	6.51[d]	Phosphate	80°	18.6
	6.57[e]	Phosphate	80°	21.4
	7.21	Phosphate	80°	84.5
	9.22	Carbonate	80°	8350
	1.03	HCl	60°	855
	9.26	Carbonate	60°	1331
	1.20	HCl	30°	42.7
	9.31	Carbonate	30°	35.3
IV	1.1	HCl	80°	302
	3.10	Formate	80°	2.0
	4.98	Acetate	80°	0.11
	7.05	THAM	80°	<0.05[f]

[a] Buffer concentration 0.025 M except for HCl (0.1 M). [b] Contains 10^{-3} M Fe^{3+}. [c] Contains 10^{-3} M Cu^{2+}. [d] Contains Fe^{3+}. [e] Contains Cu^{2+}. [f] No degradation was observed after 104 days at 80°.

and potassium chloride was added to make the ionic strength 0.10 (0.20 for solutions of IV). Metal ions (10^{-3} M) were added to some buffer solutions; however, precipitation occurred with both iron and copper at pH 6.5. These solutions were filtered at room temperature, and the rate constants were thus measured in solutions saturated with these metals.

The pH of all solutions was measured at the reaction temperature (30, 60, or 80°)[3]. Reaction solutions were typically prepared by adding 4 ml of a 2.5-mg/ml methanol solution of III to 96 ml of buffer to give a final solution containing 100 μg/ml of III and ~4% methanol. The solutions were stored in sealed amber glass ampules and placed in a constant-temperature water bath at 80, 60, or 30° for the appropriate period of time. They were then removed, and in the case of the 30° samples the ampules were frozen until they were assayed. The 80 and 60° samples were refrigerated (4°) until assayed. The solutions were then diluted 2:5 with mobile phase and analyzed by HPLC for the amount of III (or IV) remaining compared with an initial sample kept at 4°.

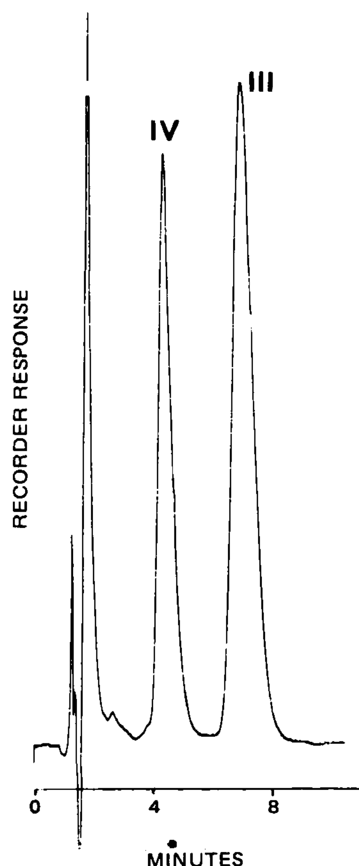

Figure 1—*Chromatogram of an aqueous solution of III after partial hydrolysis to IV at pH 1.03, 60°.*

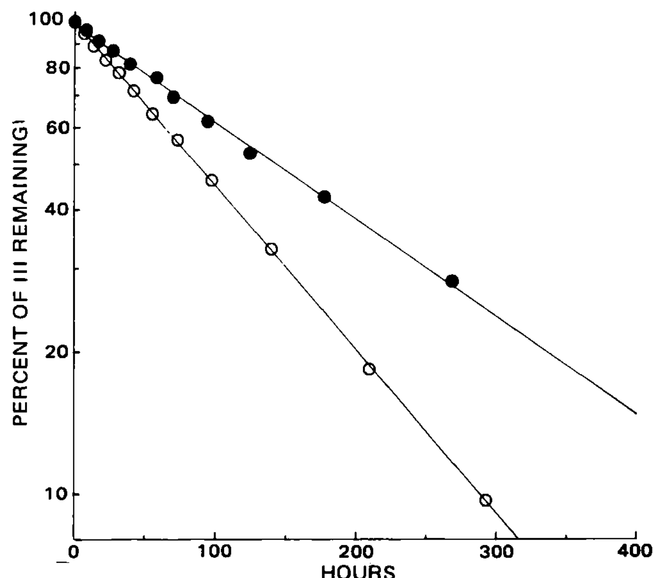

Figure 2—*First-order plot for the degradation of III at 60° in a pH 9.26 buffer solution (○) and a pH 1.03 buffer solution (●).*

HPLC Method—An HPLC system consisting of a pump[4], a variable-wavelength detector[5] and a 100-μl fixed-loop injector[6] was used. A 10-μm ODS column[7] was used with a mobile phase of methanol–0.02 M acetic acid (55:45) and detection wavelength of either 270 or 219 nm to achieve baseline separation of compounds III and IV (Fig. 1). Quantitation by peak area integration[8] gave excellent linearity for III over the range of concentrations investigated in the kinetics. The mobile phase in the above method was modified slightly (45:55 ratio of methanol to 0.02 M acetic acid) to follow the degradation of IV.

RESULTS AND DISCUSSION

The rate of disappearance of fenprostalene (III) in aqueous solution was followed using HPLC. At 80° the reaction was studied over the pH range 1.1–9.2, while at 30 and 60° the reaction was studied only at the extremes of this pH range. The degradation reaction obeyed pseudo-first-order kinetics as evidenced by the linear log percent remaining *versus* time plot shown in Fig. 2 for pH 9.26 and 1.03 at 60°. The observed pseudo-first-order rate constants (k_{obs}) are listed in Table I.

Degradation of III throughout the entire pH region was found to give the corresponding acid (IV) of fenprostalene as the only observable product by HPLC (Fig. 1). Mass balance was determined by comparing the combined area of the peaks representing III and IV throughout the time course of the reaction with the initial area of III. Greater than 94% mass balance was found for both acid- and base-catalyzed decomposition of III by this method. These results are consistent with hydrolysis of the ester group at C-1 as the exclusive reaction occurring in aqueous solution.

The log k_{obs} *versus* pH profile for the hydrolysis of III in aqueous solution at 80° is shown in Fig. 3. The line drawn through the experimental points represents the rate law described in Eq. 1 where $k_{H^+} = 3.94 \times 10^{-3}$ M^{-1} sec^{-1} and $k_{HO^-} = 2.16$ M^{-1} sec^{-1}:

$$k_{obs} = k_{H^+}[H^+] + k_{HO^-}[HO^-] \quad \text{(Eq. 1)}$$

This simple rate law contains only two terms: a specific acid term and a specific base term. The excellent fit of the theoretical line to the data

[3] Radiometer model PHM 64 pH meter equipped with a Radiometer model GK2401C electrode.

[4] Altex model 110 A.
[5] Cecil model CE 212.
[6] Rheodyne model 70-10.
[7] Spherisorb ODS particles (10 μm) were packed under high pressure in a 250 mm × 3.2-mm i.d. column.
[8] Spectra-Physics model SP-4000.

Table II—Second-Order Rate Constants for the Acid- and Base-Catalyzed Decomposition of III [a]

Temperature	$k_{H^+} \times 10^5$, M^{-1} sec^{-1}	k_{HO^-}, M^{-1} sec^{-1}
80°	394	2.16
60°	81.0	0.761
30°	6.72	0.118

[a] Calculated from the data in Table I.

indicates no other catalytic terms are necessary to explain the observed data. This also implies that it is likely that no significant buffer catalysis occurred in these solutions. It is also apparent from Fig. 3 that the presence of Cu^{2+} and Fe^{3+} had no effect on the rate of degradation of III.

The rate equation describing the pH dependence for the degradation of III (Eq. 1) is likely to hold at low temperatures as well as at 80°. The second-order rate constants k_{HO^-} and k_{H^+} at 60 and 30° were thus obtained by determining k_{obs} at high pH (9.3) and low pH (1.1) and calculating k_{HO^-} and k_{H^+} from Eqs. 2 and 3, respectively:

$$k_{HO^-} = \frac{k_{obs}[H^+]}{K_w} \quad \text{(Eq. 2)}$$

$$k_{H^+} = \frac{k_{obs}}{[H^+]} \quad \text{(Eq. 3)}$$

The second-order rate constants at 80, 60, and 30° are shown in Table II and were used to calculate the activation energies for the specific acid- (17 kcal/mole) and base-catalyzed (12 kcal/mole) hydrolysis of III[9].

The degradation kinetics of IV were also investigated at 80° from pH 1 to 7. The degradation reaction followed pseudo-first-order kinetics, and the observed rate constants obtained are shown in Table I. From the plot in Fig. 3 one can see that the degradation rate of IV is acid catalyzed and is significantly slower than that of its methyl ester (III) in the acidic pH region. The degradation rate for IV at pH 7.0 was too slow to measure. If the assumption is made that the degradation reaction of IV does not involve the C-1 carboxyl group[10], then this acid-catalyzed pathway is probably available to fenprostalene (III) itself. Hydrolysis of the C-1 methyl ester, however, is preferred by ~10-fold over this alternate degradation pathway for III at 80° in the acidic pH region.

[9] These results are nearly identical to those reported for the hydrolysis of ethyl acetate (7).

[10] Jones *et al.* (3) studied the degradation of II in acidic solution under aerobic conditions. The products were a complex mixture of compounds, and no major products were identified. We have not identified the degradation products of IV in acidic media but have observed on HPLC that several products are formed.

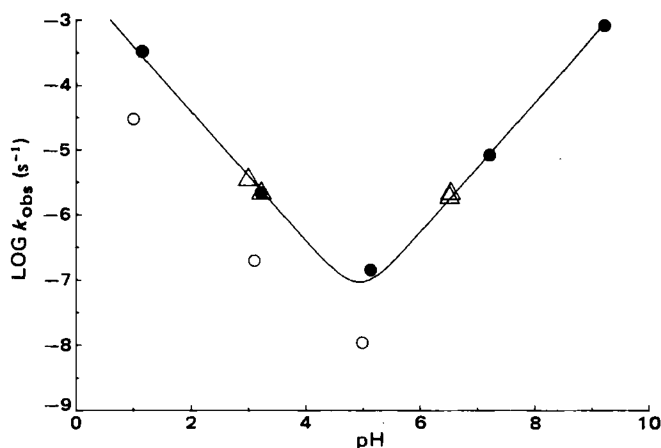

Figure 3—*Log* k_{obs} versus *pH profile for the hydrolysis of III at 80°. Key: solid circles represent simple buffer solutions, open triangles represent buffer solutions containing* Cu^{2+} *and* Fe^{3+} *ions, and the reaction of IV in aqueous solution at 80° is shown by the open circles.*

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Determination of Hydrazine in Pharmaceuticals III: Hydralazine and Isoniazid Using GLC

F. MATSUI ×, **D. L. ROBERTSON, and E. G. LOVERING**

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Abstract □ A GLC procedure has been developed for the determination of hydrazine in hydralazine and isoniazid drug raw materials, single and multicomponent tablets, injectables, and syrups. The method is based on the derivatization of hydrazine with benzaldehyde to form benzalazine. The minimum detectable amount of hydrazine in hydralazine and isoniazid raw materials and formulations is ~0.0003%. No hydrazine was found in the hydralazine raw material specimens examined. Traces of hydrazine (~0.0003%) were found in some tablet lots and ~0.02% was found in an injectable product. A trace of hydrazine was found in one lot of isoniazid raw material and low levels (0.0012 and 0.0029%) were found in isoniazid tablet products. An isoniazid syrup contained ~0.2% hydrazine.

Keyphrases □ Hydrazine—determination in hydralazine and isoniazid by GLC □ Hydralazine—GLC, determination of hydrazine □ Isoniazid—GLC, determination of hydrazine □ GLC—determination of hydrazine in pharmaceutics, hydralazine, isoniazid

Previous papers in this series described high-performance liquid chromatographic (HPLC) methods for the determination of hydrazine in isoniazid and phenelzine products and reported typical amounts found in commercial formulations (1, 2). Since hydrazine is a mutagen (3) and a carcinogen in laboratory animals (4), concern over its presence in drug products resulted in proposed regulatory action in the United States (5) and to the recall of

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