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Research Papers

THE STABILITY OF SOME ARYLOXY PROSTAGLANDIN ANALOGUES

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SUMMARY

Methods based on HPLC, or TLC of ^{14}C-labelled compounds were developed to study the stability of solutions of two aryloxy analogues of $PGF_{2\alpha}$ in a series of experimental buffer solutions. At pH values greater than 5 no appreciable decomposition occurred even after prolonged storage at 100°C. At pH values below 4 two lactones were slowly formed, and phenols were slowly formed by a specific acid catalysed reaction sequence. No difference in rate was observed between the two compounds.

Storage under oxygen did not affect the stability at pH values greater than 5, but at lower pH an acid-catalyzed oxidation occurred, causing scission of a double bond and oxidation of the aromatic ring. The m-trifluoromethylphenoxy analogue was more stable to oxidation than the m-chlorophenoxy analogue.

INTRODUCTION

Wide differences are observed between the stabilities of PGF and PGE compounds in aqueous solution, because of the presence of the β-ketol system in the latter series. These differences were first demonstrated by Karim et al. (1968); PGE_2 was unstable under alkaline conditions whereas $PGF_{2\alpha}$ was very stable except under strongly acid conditions.

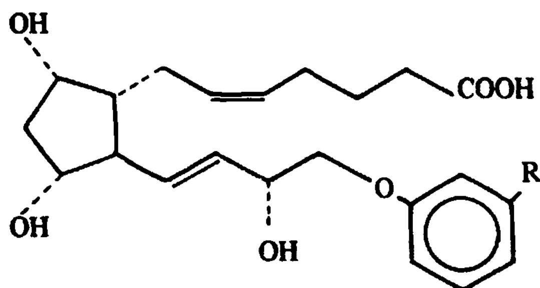
Although since then the stability of the E series has been extensively studied, for example by Andersen (1969), Nugteren et al. (1966) and Monkhouse et al. (1973), published investigations of the stability of PGF compounds are few. Roseman et al. (1973) showed that $PGF_{2\alpha}$ tromethamine salt was unchanged in aqueous solution after one week at 47°C. Roseman et al. (1976) stated that the 15-epimer is a major decomposition product in acidic, buffered solutions of $PGF_{2\alpha}$ and that dehydration also occurred.

The purpose of the present study was to develop analytical methods which would elucidate the degradation pathways of a pair of aryloxy analogues of $PGF_{2\alpha}$ in kinetic terms, and to identify the products of their decomposition, where possible.

Preliminary experiments demonstrated that, while under the neutral conditions that are used for pharmaceutical formulation their decomposition is almost immeasurably slow, these compounds form novel reaction products in acid (pH < 5), the kinetics of whose formation possesses some unusual features. This paper is confined to a consideration of these acid-catalyzed reactions.

MATERIALS AND METHODS

The compounds studied are fluprostenol (I) and cloprostenol (II), a pair of prostaglandin analogues used as luteolytic agents in farm animals.



I, R = CF_3
II, R = Cl

Radiochromatographic assay

Five decomposed samples and a sample of the initial solution which had been stored at 4°C were generally developed together on a TLC plate (Machery-Nagel Chromatoplate, silica gel GF_{254}, 0.25 mm) using 40 μl volumes of each. Each sample would initially have contained 600 μg of the prostaglandin sodium salt labelled at the carboxyl carbon atom with ^{14}C (specific activity about 0.5 μCi mg^{-1}). After single development in toluene : dioxan : glacial acetic acid (20 : 20 : 1) the solvents were removed in a warm air stream. The chromatograms were visualized using iodine vapour and irradiation at 254 nm. They were then subjected to contact autoradiography and, after comparison with the autoradiogram, marked into the relevant zones for subsequent cutting. The activity of each zone was determined using an Intertechnique SL30 scintillation counter, (at 4°C in naphthalene : dioxan : Butyl-PBD fluor) and with an off-line computer programme the dpm of each zone was calculated using external standard channels ratio quench correction. The proportion of total chromatogram activity residing in each zone was calculated by normalization.

High performance liquid chromatography (HPLC) assay

To 100 μl of the sample solution was added 100 μl of an aqueous solution of sodium 4-hydroxy-n-propylbenzoate as internal standard, following which 5 μl samples were chromatographed using a 100 × 0.2 cm i.d. column packed with Permaphase ODS

(Dupont). The eluting solvent was 0.04 M boric acid + 7% methanol adjusted to pH 6.6 with 0.1 N sodium hydroxide solution and used at a flow rate of 0.6 ml min^{-1}. Detection was by UV absorption at 220 nm. The instrument used was a Dupont Model 830 liquid chromatograph.

Alternating injections of the initial solution which had been stored at 4°C and treated similarly with internal standard were made. The peaks obtained were well separated and could be estimated by calculation of the peak height ratio of prostaglandin to internal standard. Response curves of micrograms prostaglandin to peak height ratio were linear over the range studied. The presence of a peak corresponding to the phenol decomposition product (m-trifluoromethyl phenol in the case of I, m-chlorophenol in the case of II) allowed the amount of decomposition to this product to be determined by use of calibration graph of peak height: micrograms phenol chromatographed. Again, linear calibration graphs were obtained.

Kinetic studies

These were carried out in acetate : borate : phosphate experimental buffer system (Britton, 1955) at nominal pH values of 2–5.

A suitable amount of (I) or (II) labelled with ^{14}C at C_1 (specific activity about 0.5 μCi mg^{-1}) was dissolved in the buffer solution to give a concentration of about 0.05%, the solution apportioned into ampoules and sealed under oxygen or nitrogen, and stored in an incubator at 100°C. A little of the solution was retained, stored at 4°C and used as initial sample.

HPLC assay of samples from a kinetic run showed a small interference by a minor secondary decomposition product unresolved from the TLC zone of the parent prostaglandin. Significant error was only noted under conditions of high decomposition (<30% remaining).

For this reason kinetic data at the lower pH values were obtained using the HPLC assay. In these experiments solutions containing about 0.03% of the unlabelled (I) or (II) in the same buffer solutions were used.

Identification of the decomposition products

Preliminary experiments followed by TLC quickly established that distribution of R_f values of the isolated reaction mixtures was the same for each of the compounds. This strongly implied that, apart from the aromatic substitution, structures to be elucidated were common to each.

In order to identify the products of decomposition in the absence of oxygen, 70 mg (II) was dissolved in 10 ml of the acetate–borate–phosphate buffer system at pH 2.5 in a 10-ml ampoule. The mixture was degassed with, and sealed under argon and stored at 100°C for 13 days. The organic material was extracted by the serial use of chloroform, diethyl ether and n-butyl acetate. The organic layers were combined, dried (sodium sulphate/magnesium sulphate), and evaporated to dryness. The mixture was separated into four major components by preparative TLC on 1 mm layers of silica gel PF_{254} (E. Merck and Co., Darmstadt) developed in toluene : dioxan : glacial acetic acid (20 : 20 : 1). Analytical TLC and HPLC examination using a Cecil CE210 pneumatic coil pump and CE212 variable wavelength ultraviolet monitor under the following conditions were

used to monitor the purity of the separated fractions. The column (20 × 0.8 cm i.d.) was packed with 5 μm diameter silica gel (Partisil-5, Whatman Labsales Ltd.) and the eluting solvent was hexane : ethanol : orthophosphoric acid (850 : 150 : 2) at a flow rate of 0.8 ml min^{-1}. Detection was by UV absorbance at 225 nm.

After repurification where necessary the fractions were examined by mass spectrometry using a Dupont 491B low resolution double focussing instrument and by Fourier Transform NMR on a Bruker W90 90 MHz spectrometer. Direct probe insertion mass spectra of the TMS ester/TMS ether derivatives were obtained by probe temperature programming from 30 to 200°C. NMR spectra were of limited value due to interference from solvent impurities, and are not reported in detail.

To identify the oxidation products, 170 mg (II) as its sodium salt were dissolved in 30 ml of the acetate–borate–phosphate buffer system at pH 4.3 and distributed between three 20 ml ampoules which were sealed under oxygen and stored at 100°C for 20 days. Extraction as before yielded the decomposition product mixture which was dissolved by

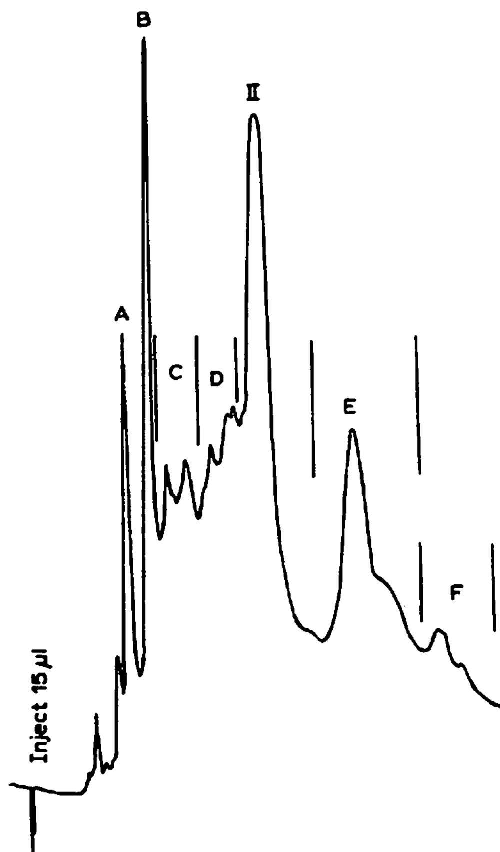


Fig. 1. Location of fractions A–F in preparative HPLC chromatogram of (II) after decomposition under oxygen. Column, 20 × 0.8 cm i.d. 10 μm silica gel (Partisil-10); eluting solvent, hexane : ethanol : orthophosphoric acid (850 : 150 : 2); flow rate, 1.3 ml min^{-1}; monitoring wavelength, 225 nm.

warming in 400 μl alcohol. After analytical TLC 20 μl aliquots were subjected to preparative HPLC separation.

A typical chromatogram is shown in Fig. 1. Each of the 6 fractions shown was collected from each injection, pooled and after addition of water isolated by ether extraction. The purity of the separated fractions was monitored by analytical HPLC, and after further preparative HPLC purification where necessary the fractions were examined by mass spectrometry. Mass spectra of the methyl ester/TMS ether derivatives were obtained by direct probe insertion as before, or by gas chromatography/mass spectrometry using a 1000 cm × 0.6 cm i.d. column packed with 2% SE-30 on Gas-Chrom Q (80–100 mesh), at a temperature of 220°C, with helium carrier flow rate of 50 ml min^{-1}.

RESULTS

Kinetic studies

Both compounds were found to be extremely stable to both hydrolysis and oxidation at pH values greater than 5, the region of importance in pharmaceutical formulation. Under very low pH conditions, however, both hydrolytic and oxidative degradations could be induced by heating at 100°C.

As expected the non-oxidative degradation followed first-order kinetics over the pH range studies. The order of reaction under oxygen was, however, obscure. Reasonable linearity was obtained by plotting the data graphically under either zeroth or first order conditions. Calculation of the % fit of these results to the expressions $y = a + bx$ (zeroth

TABLE 1

FIRST-ORDER RATE CONSTANTS ($k_{obs} \times 10^2$ in $days^{-1}$) FOR THE DECOMPOSITION OF I AND II IN AQUEOUS SOLUTION AT 100°C

Compound	pH	Under N_2		Under O_2		
		$k_{obs} N_2 \times 10^2$	% standard error of data fitting	$k_{obs} O_2 \times 10^2$	% st. ndard error of data fitting	$k_{O_2} \times 10^2$
(I)	2.2	26.9	9.5	–	–	–
	2.45	26.8	2	–	–	–
	2.6	15.1	9.7	35.9	4.7	20.8
	2.8	10.8	4.8	–	–	–
	3.0	4.6	17.4	–	–	–
	3.6	3.2	7.8	–	–	–
	4.3	0.75	17.3	6.7	8.2	5.95
	4.9	0.42	26	4.6	6.5	4.2
(II)	2.3	35.9	3.9	–	–	–
	2.6	17.2	9.1	56.8	8.6	39.6
	4.2	1.4	10.7	12.0	17.5	10.6
	4.7	0.44	15.9	5.0	7.6	4.5

order assumptions) and to $y = ae^{bx}$ (first-order assumptions), showed that in general a better first-order fit was obtained. Therefore, rate constants at 100°C on first-order assumptions were calculated using a least squares analysis computer program. These are shown in Table 1 together with % standard error of first-order fitting.

Experiments not reported here showed that the rate of decomposition at still higher pH values was too low for meaningful values to be obtained due to the diminishing precision of rate constant determination for these very slow reactions.

Use of the Student's t-test showed that under oxygen, (II) decomposed significantly faster than (I) ($t_{calc} = 2.23$ at 5% level, t_{exp} at pH 4.2 = 2.53). Under nitrogen, however, a significant difference was observed at pH 2.2–2.3, at all higher values differences in the reaction rates being indistinguishable (at pH 2.2–2.3 $t_{calc} = 2.26$, $t_{exp} = 3.03$; at pH 2.6 $t_{calc} = 2.23$, $t_{exp} = 0.99$).

The rate constants for the first-order oxidation process $k_{O_2} = k_{obsO_2} - k_{obsN_2}$ shown in Table 1 clearly indicate an unexpected acid catalysis. The log k_{O_2} : pH relationship for both compounds has a gradient very much less than 1.0 which must indicate either that the first order assumption is invalid or that the oxidation process is complex, its nature varying with pH.

Fig. 2 shows the log k : pH relationship for the anaerobic reaction. Below pH 3.5 the rate is approximately linear in H^+.

$$k_{obs(N_2)} = k_1 H^+ + k_2 \left(\frac{H^+}{H^+ + K_a} \right) \quad (1)$$

The line shown illustrates a reasonable fit to the data points and is calculated using Eqn. 1, by substitution of the values: $k_1 = 63$ M^{-1} $days^{-1}$ (by extrapolation of the log k : pH relationship to pH 0, i.e. to unit hydrogen ion concentration), $pK_a = 4.5$

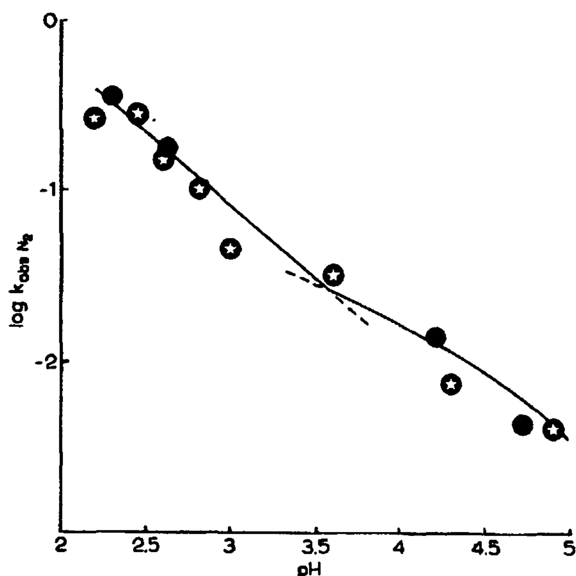


Fig. 2. pH profile of non-oxidative decomposition in buffered aqueous solution at 100°C. ✪, (I); ●, (II). Line calculated according to Eqn. 1 (see text).

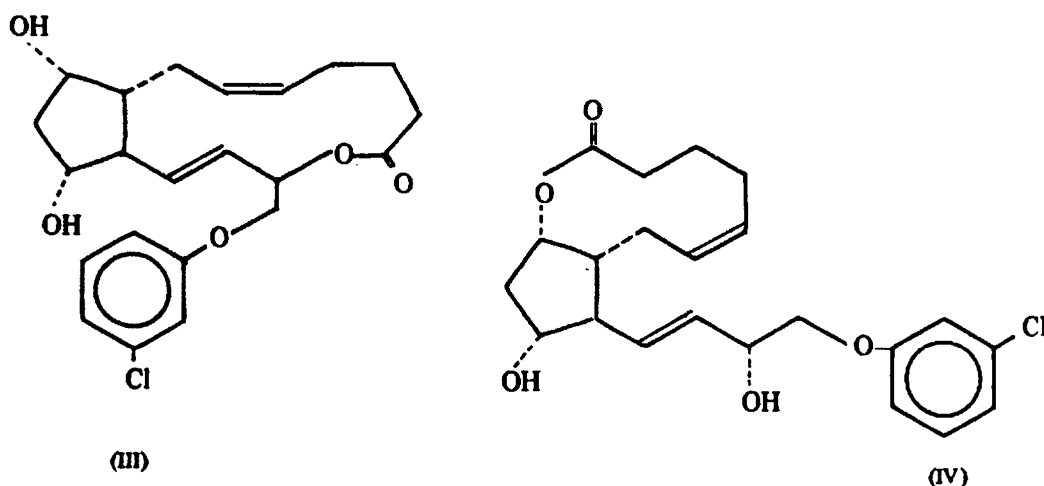
(within the range normal for aliphatic carboxylic acids; measurement of pK_a for (I) and (II) is precluded by their low aqueous solubility) and $k_2 = 0.013$ days^{-1} (by inspection).

The decomposition products

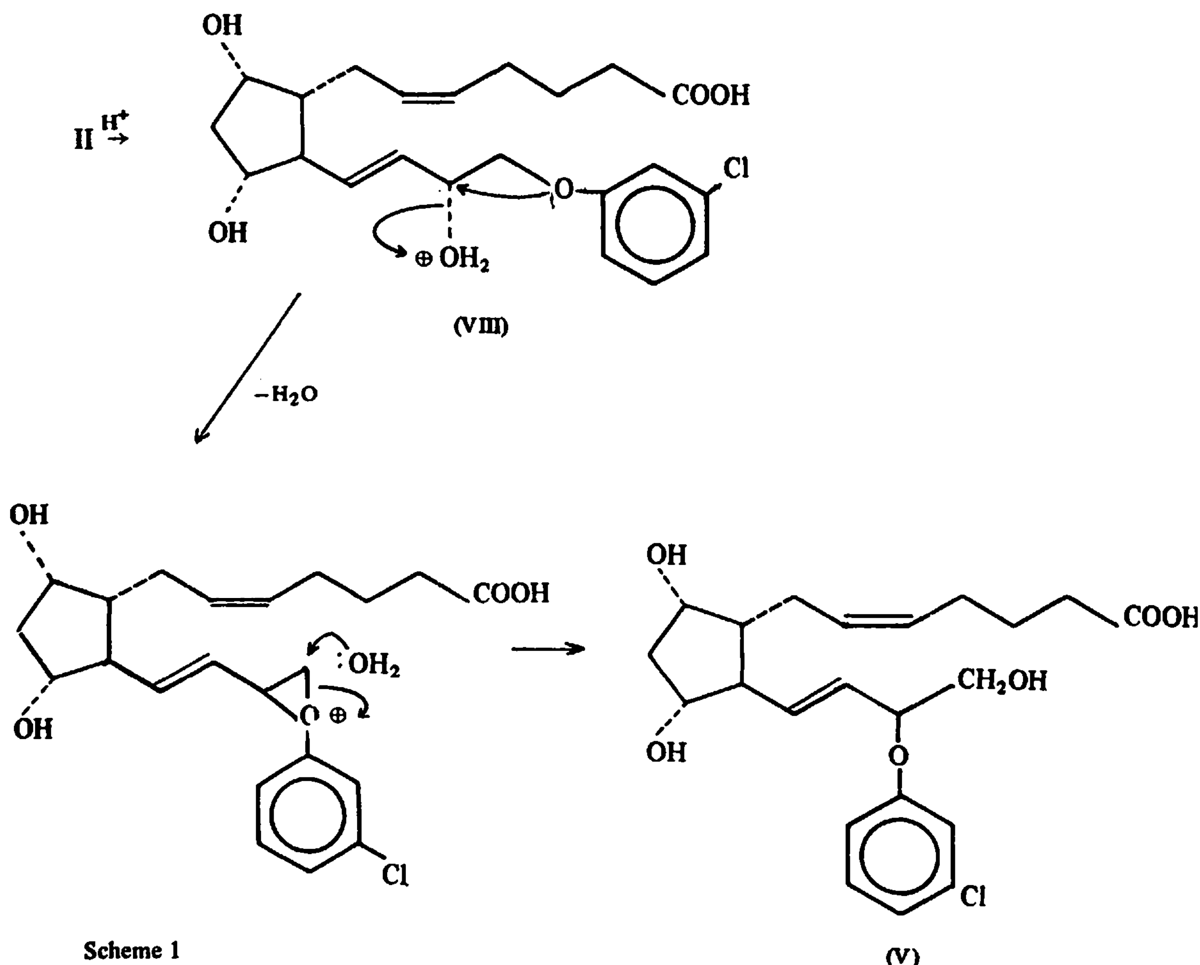
After anaerobic decomposition of (II) four major fractions were isolated, as described previously. The least polar of these ($R_f = 0.71$) was identified by comparison of R_f value and HPLC retention time as m-chlorophenol.

Two of the remaining three fractions ($R_f = 0.38$ and 0.49) gave identical mass spectra (as trimethyl silyl derivatives) with $M^+ = 550$. The occurrence of only two serial losses of m/e 90 showed the presence of only two hydroxyl groups. Strong M-141 ions were probably due to loss of $CH_2C_6H_4Cl$ as occurs in the parent compound, and the series of ions (M-141-90, M-141-2 x 90) corresponding to silanol losses again showed the lower side-chain to be essentially intact. The spectra were consistent with those predicted for a lactone formed by dehydration. A spectrum of an authentic sample of the 1,9 lactone was identical to the above. In the proton NMR spectrum of the $R_f = 0.49$ fraction the cis olefinic resonances were shifted upfield, as was also shown by the spectrum of the 1,9 lactone.

Corey et al. (1975) have reported a facile synthesis of prostaglandin lactones in which the 1,9 and the 1,15 isomers only are formed. The strained 11-membered ring 1,11 lactone is not produced. It seems likely, therefore, that the $R_f = 0.38$ and $R_f = 0.49$ fractions are the 1,15 and 1,9 lactones (III) and (IV).



The remaining fraction ($R_f = 0.19$) gave a mass spectrum superficially the same as II, ($M^+ = 712$, 4 –OH groups denoted by 4 serial losses of m/e 90), but the different chromatographic behaviour together with the ɔlacement of the expected M-141 by M-127 indicated isomerization in the lower side-ɔnain. The most likely structure consistent with the spectrographic data is the isomer (V) formed by the acid-catalyzed 1,2-shift shown in Scheme 1.



Scheme 1

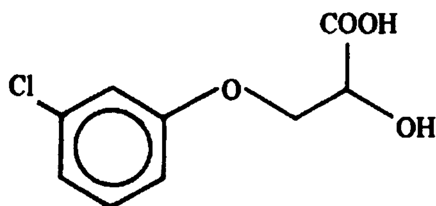
(V)

Other isomeric structures are of course possible.

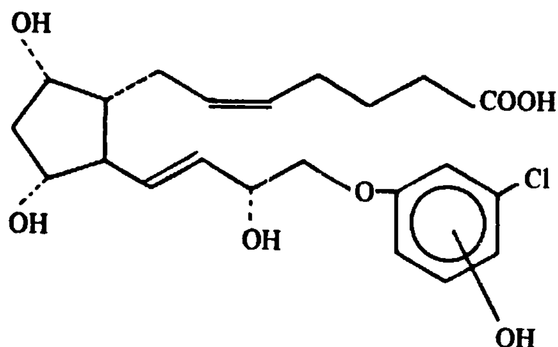
The identification of the products of oxidative decomposition proved more difficult. The major product, which on TLC under the conditions used throughout the study gave a spot of $R_f = 0.05$ strongly fluorescent at 366 nm, did not survive HPLC, and was similarly unisolable by preparative TLC, This instability suggests a peroxidic reaction product.

In Fig. 1, Fraction A was identified by comparison of retention time and mass spectrum as m-chlorophenol. Fraction B gave a mass spectrum containing the strong fragments m/e = 287, 277 (probably M-CH_3 and M-CH_3-$COOCH_3$) and only one ion (m/e = 197) corresponding to silanol loss. These ions had isotope patterns consistent with monochlorination. The spectrum was consistent with that expected for the hydroxy-acid (VI).

Fraction F gave a mass spectrum with $M^+ = 684$ and strong, monochlorinated fragments at m/e = 669 and 579 (M-CH_3 and M-CH_3-90). No M-141 fragment was observed but an ion at M-171 possibly from loss of $CH_2OPh(Cl)(OCH_3)$ was apparent. This is consistent with this fraction being the hydroxyl substituted derivative (VII).



(VI)



(VII)

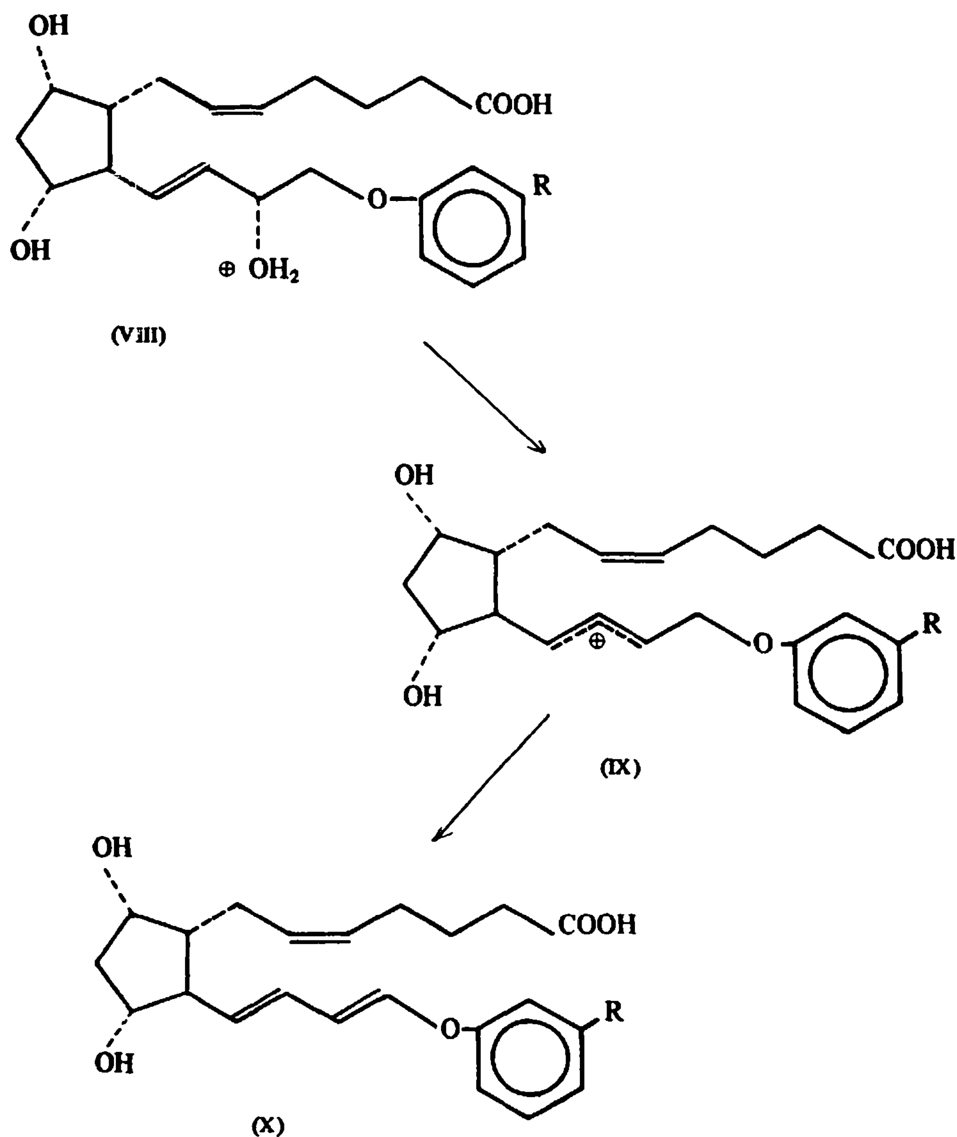
Fractions B–D gave uninterpretable spectra, presumably due to their multicomponent nature.

DISCUSSION

It seems unlikely that the formation of the relevant phenol, shown to be a major route of non-oxidative decomposition, occurs through the well-known A-1 acid-catalyzed hydrolysis of the aryl–alkyl ether, much more aggressively acid conditions generally being required (Burwell, 1954). However, acid-catalyzed elimination of water from the 15-hydroxyl group of the prostaglandin (VIII → IX, Scheme 2) would produce an aryl–vinyl ether (X) whose subsequent solvolysis is expected to be rapid (Rekasheva, 1968), and almost quantitative (Wood and Healy, 1970).

Liberation of the phenol by this route should be competitive with the isomerization process of Scheme 1 as both would go through the common intermediate (VIII). Loss of water in this way would be facilitated by the ease of formation of the vinyl cation (IX). The other product from loss of a phenol would be a β,γ-unsaturated aldehyde, which has not been detected, but as a labile species might well fail to survive the reaction conditions.

Lactonization reactions, e.g. of canrenoic acid, described by Garrett and Won (1971), are usually first-order and specifically acid-catalyzed. At low pH, therefore, lactonization is expected to occur in parallel with the reactions discussed above. However, there is a possible pH-independent route for lactonization, via intramolecular attack of hydroxyl on the unionized carboxyl group. At pH values at and above the pK_a value of the carboxyl group this reaction is expected to attenuate as the availability of the neutral species



Scheme 2

diminishes, and perhaps through competitive hydrolysis of the lactone formed. A superimposed reaction of this type could account for the unexpected form of the pH profile as expressed by Eqn. 1. In this equation, k_1 is a composite term due to lactonization, isomerization, and loss of phenol, whereas k_2 is due to lactonization alone. Consistent with this hypothesis, in experiments not reported here, phenol production is found to diminish as a proportion of the overall reaction process as the pH rises, and may be produced by further reaction of other components of the reacting system, i.e. the lactones (III) and (IV) and perhaps the isomer (V).

In their study of canrenoic acid, Garrett and Won (1971) found lactonization processes to be first order in hydrogen ion and in carboxylate anion, whereas in this case, hydrogen ions and the free carboxyl group are implicated. However, little is known concerning the kinetics of large-ring lactone formation, and no study has been published which is sufficiently detailed to afford a comparison.

In the absence of detailed information concerning its products, little can be said concerning the oxidation process. It is at first sight curious that this too should be acid-catalyzed. However, there are a number of ways in which hydrogen ions might aid oxidation, perhaps via reversible protonation at or near a double bond to give an electron-deficient species which would then be highly susceptible to attack by oxygen [1].

The identification of two lactonization processes in the acid-catalyzed decomposition of (I) and (II) suggests strongly that similar reactions are likely for $PGF_{2\alpha}$ itself. However, no such reactions have yet been reported, and as no detailed kinetic investigations of $PGF_{2\alpha}$ decomposition have yet appeared, speculation as to the relative rates of these reactions would be premature.

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[1] We are indebted to Professor J.B. Stenlake, University of Strathclyde, for this suggestion.

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