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Prostaglandin Prodrugs. 5.[1] Prostaglandin E_2 Ethylene Ketal

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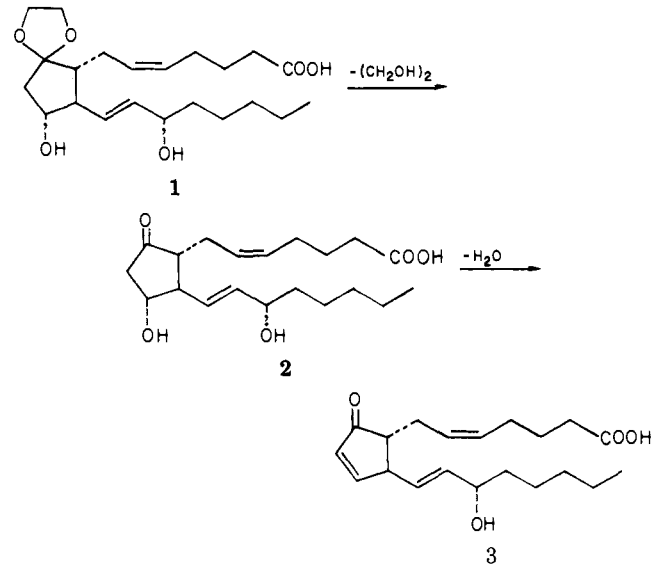
In order to improve the chemical stability of prostaglandin E_2 (2), prostaglandin E_2 ethylene ketal (1) was prepared by direct ketalization of 2 with ethylene glycol in benzene. To establish a quantitative assessment of 1 as a chemically stable and orally active prodrug of 2, the hydrolysis of 1 to 2 and the subsequent dehydration of 2 to prostaglandin A_2 (3) were followed at 25 °C and six pH's ranging from 2.0 to 6.5 by means of a high-pressure liquid chromatographic procedure. Kinetic results clearly indicate that 1 should be quantitatively hydrolyzed back to the parent drug 2 under the stomach conditions without loss to 3. At pH 2 and 25 °C, the half-lives of the hydrolysis of 1 to 2 and dehydration of 2 to 3 are in the order of 1 h and 14 days, respectively. The preliminary data on the biological response after oral administration of 1 appeared to indicate that 1 is bioequivalent to 2.

The naturally occurring E and F prostaglandins have found a wide clinical application in human reproduction. For instance, prostaglandin E_2 (2, PGE_2) has been successfully used for labor induction and termination of pregnancy.[2] However, like most β-hydroxy ketones, the E series prostaglandins readily undergo dehydration to produce the A series prostaglandins[3] which have different spectra of biological activity.

β-Oxy cyclic ketones with various leaving groups of pK_a ranging from 5 to 16 including OH^- as in the case of 1 are known to undergo β-elimination through the E1cB mechanism,[4] and thus the dehydration of 2 is believed to occur in the same manner with either the formation of enolate at C-9 or the expulsion of OH^- (or H_2O) from C-11 as the rate-determining step. Energetically, the fundamental driving force for the dehydration appears to be the reduction in free energy content derived from an extended conjugation present in prostaglandin A_2 (3, PGA_2). Therefore, any derivatives saturating the carbonyl group at C-9 of 2 should be prodrugs with excellent stability so long as they are converted to 2 with ease in the biological environment. For example, it was claimed that the reversible nucleophilic addition of bisulfite ion across the C-9 carbonyl group of 2 can improve the stability in aqueous solutions of neutral pH's.[5] Under acidic conditions the bisulfite adduct dissociates to rapidly release the parent prostaglandin.

In the present paper, we would like to report the synthesis of the C-9 ethylene ketal of 2, which was found to be stable in the solid state, and its conversion to the parent compound 2 under acidic conditions, similar to the conditions encountered in the stomach. Implication is the possible use of 1 for an oral dosage form of 2. To the present authors' knowledge, there are no enzymes in the blood stream which can effectively hydrolyze 1 to 2, and, hence, the use of 1 in developing a parenteral solution of 2 appears to be of a remote possibility. Since both hydrolysis of 1 to 2 and dehydration of 2 to 3 are catalyzed by specific acid, the availability of 2 in the GI tract and ultimately in the blood stream will critically depend on the relative magnitude of both rate constants, k_1 for 1 to 2 hydrolysis and k_2 for 2 to 3 dehydration. For instance, if k_2 is much larger than k_1, the concentration of 2 will never be built up in the GI tract. The kinetics of 1 → 2 → 3 (Scheme I) was followed using a high-pressure liquid chromatographic (HPLC) procedure to establish a quantitative assessment of 1 as a chemically stable and orally active prostaglandin prodrug.

Scheme I



Experimental Section

Synthesis of PGE_2 Ethylene Ketal (1) (2 → 1). A mixture of 1 g of PGE_2, 20 mL of freshly distilled ethylene glycol, and 100 mL of benzene was heated at reflux under nitrogen with vigorous stirring for 24 h. The reaction mixture was cooled to room temperature, diluted with water, and extracted thoroughly with ethyl acetate. The combined organic layer was washed with water and brine, dried over sodium sulfate, and evaporated in vacuo. The crude product, 1.3 g, was taken up in 50 mL of methanol and treated under nitrogen with 20 mL of 3 N aqueous potassium hydroxide. [This conversion of unreacted PGE_2 to PGB_2 via PGA_2 (3) simplifies an otherwise very difficult chromatographic separation of PGE_2 and its ketal.] The mixture was allowed to stand for 2 h at room temperature and was then concentrated in vacuo to remove most of the methanol. The residue was diluted with ice and water, acidified with 35 mL of cold 2 N aqueous potassium bisulfate, and extracted thoroughly with ethyl acetate. The combined extracts were washed with water and brine, dried over sodium sulfate, and concentrated in vacuo. The 1.2-g residue was chromatographed on 90 g of Mallinckrodt CC-4 silica. Elution proceeded as follows (10-ml fractions): 500 mL of 50% ethyl acetate–Skellysolve B, fractions 1–50; 500 mL of 65% ethyl acetate–Skellysolve B, fractions 51–100; 500 mL of 80% ethyl

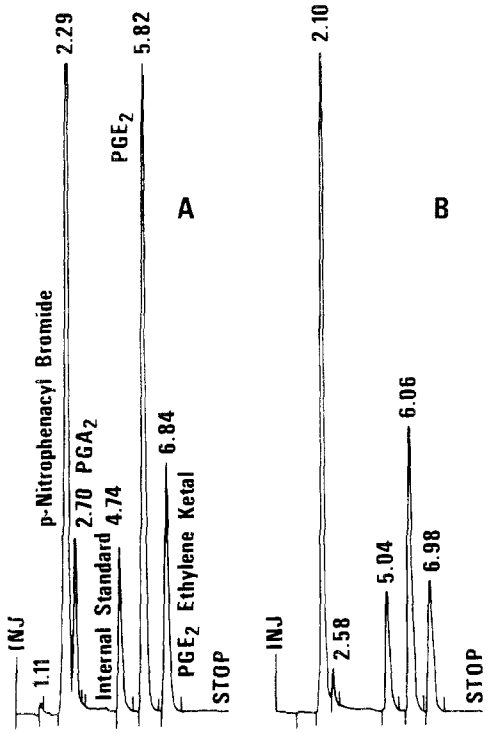


Figure 1. HPLC separation of PGE_2 ethylene ketal, PGE_2, and PGA_2 in a synthetic (A) and reaction (B) mixture. Total prostaglandins and medrol acetate (internal standard) injected are approximately 2.16 and 1.73 μg, respectively. See text for the HPLC conditions. Retention time in minutes is printed in the figure.

acetate–Skellysolve B, fractions 101–150. Fractions 101–132 were combined on the basis of TLC homogeneity and afforded 330 mg of PGE_2 ethylene ketal which crystallized on standing. Recrystallization from ether–hexane yielded 250 mg (22% of theory) of pure PGE_2 ethylene ketal, which exhibited mp 68.0–70.8 °C: ν_{max} 3300, 2700, 1730, 1315, 1240, 1215, 1165, 1145, 1070, 1035, and 970 cm^{-1}; $\nu_{max}^{basic\ EtOH}$ no detectable absorption at 278 nm. Anal. Calcd for $C_{22}H_{36}O_6$: C, 66.64; H, 9.15. Found: C, 66.46; H, 9.02.

Hydrolysis Kinetics. Throughout the experiments, the buffer concentration was maintained at 0.1 M and the total ionic strength was adjusted to 0.10 M with KCl. For the experimental pH range 2.0–6.5, HCl, $ClCH_2COOH$, CH_3COOH, succinic acid, and phosphoric acid buffers were employed. Hydrolysis was followed at 25 ± 0.5 °C, with an initial concentration 0.25 mg/mL (6.31×10^{-4} M) of 1 for the pH range 2.0–3.5 and 0.50 mg/mL (12.61×10^{-4} M) for the pH range 4.5–6.5. An aliquot of sample solution containing 1.0 mg of prostaglandins was withdrawn with proper time intervals and extracted with an equal volume of EtOAc. For samples at pH 5.50 and 6.50, the extraction was carried out after the solution was acidified. Exactly a half volume of the EtOAc layer was then transferred to a 3-mL vial and the solvent was evaporated to dryness under N_2 stream at room temperature.

p-Nitrophenacylation of the residual prostaglandins was carried out by adding 35 μL of 25 mg/mL *p*-nitrophenacyl bromide and 30 μL of 10 μL/mL diisopropylethylamine, both in CH_3CN.[6] After 10–15 min at room temperature, added was 20 μL of 10 mg/mL 6α-methylprednisolone 21-acetate (Upjohn Company, Kalamazoo, Mich.) in $CHCl_3$ as an internal standard. Prior to injection of the sample mixture to a high-pressure liquid chromatography (HPLC), 2.0 mL of mobile phase was also added. A high-performance silica gel column (Du Pont Zorbax Sil, 4.6 mm i.d. × 25 cm length) was used in a Du Pont Model 830 HPLC equipped with an automatic injector[7] and a Hewlett-Packard Model 3380A integrator. Other HPLC assay conditions were mobile phase = CH_3OH–CH_3CN–CH_2Cl_2 (1.5:3.5:95.0, volume ratio), pressure = 800 psi, flow rate = 1.30 mL/min, and λ = 263 nm (Du Pont Model 837 variable wavelength spectrophotometer).

A typical HPLC separation of 1, 2, and 3 is shown in Figure 1. The concentration of each compound in a mixture was determined from a calibration curve which had been constructed from a series of synthetic mixtures. Peak area ratios were used in the assay.

Table I. Rate Constants at 25 °C for the Hydrolysis of PGE_2 Ethylene Ketal (1) to PGE_2 (2), k_1, and Dehydration of 2 to PGA_2 (3), k_2

pH	k_1, s^{-1}	k_2, s^{-1}	Lit. value of k_2, s^{-1} [a]
2.08	1.93×10^{-4}		5.8×10^{-7}
2.99	2.29×10^{-5}		2.8×10^{-7}
3.50	5.78×10^{-6}	5.80×10^{-7}	2.3×10^{-7}
4.53	7.61×10^{-7}	3.36×10^{-7}	3.1×10^{-7}
5.50	1.71×10^{-7}	6.37×10^{-8}	4.0×10^{-7}
6.50	2.58×10^{-8}		5.3×10^{-7}

[a] Estimated from ref 3f.

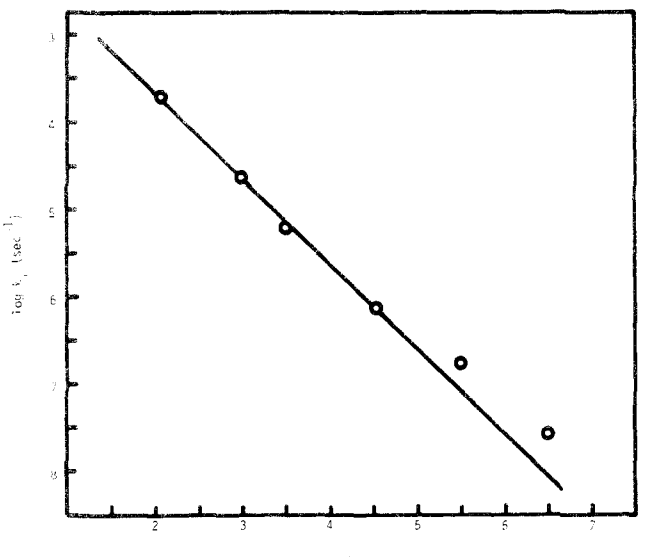


Figure 2. pH dependence of the rate constant, k_1, for the hydrolysis of PGE_2 ethylene ketal (1) to PGE_2 (2) at 25 °C. The straight line was drawn with a slope of −1.0.

Results and Discussion

Throughout the studies of hydrolysis of 1, no side reactions were observed. Since the HPLC peak corresponding to 3 was very close to the solvent front which eluted a large excess of *p*-nitrophenacyl bromide (Figure 1), the error involved in determining the concentration of 3 was much greater than others. A complete baseline resolution of 3 caused a considerably longer assay time.

For an A (k_1) → B (k_2) → C kinetic scheme, the concentration of each species at a given time is expressed below.[8]

$$A = A_0 e^{-k_1 t} \tag{1}$$

$$B = A_0 \left(\frac{k_1}{k_2 - k_1} \right) (e^{-k_1 t} - e^{-k_2 t}) \tag{2}$$

$$C = A_0 \left[1 + \frac{1}{k_1 - k_2} (k_2 e^{-k_1 t} - k_1 e^{-k_2 t}) \right] \tag{3}$$

The values of k_1 at various pH's (Table I) were determined by eq 1 over more than 2 half-life periods, and their dependence on pH at 25 °C is shown in Figure 2. A straight line with a slope of −1 on the pH–profile fits data of k_1 over a pH range 2–4.5, confirming specific acid catalyzed hydrolysis of 1, as in the case of other acetals, ketals, and ortho esters. The positive deviations of k_1 at pH 5.50 and 6.50 from the straight line are attributed to the experimental errors rather than a general-acid catalysts, not only because the concentrations of buffer components were

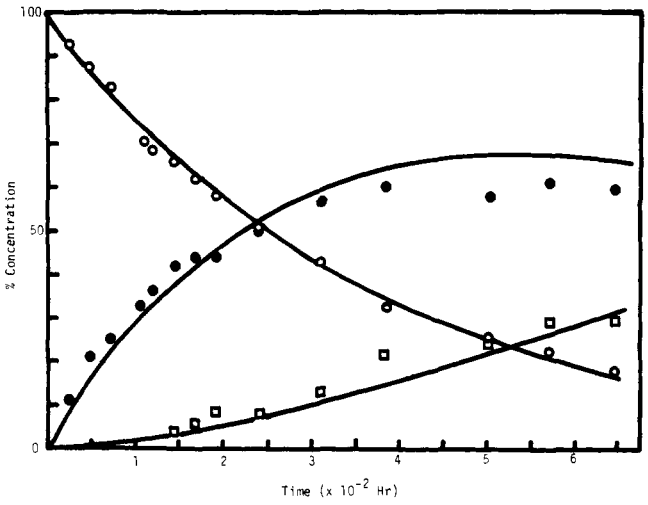


Figure 3. Concentration profiles of PGE_2 ethylene ketal (1, ○), PGE_2 (2, ●), and PGA_2 (3, □) in an irreversible consecutive reaction, 1 → 2 → 3, at 25 °C and pH 4.53. Curves were generated by substituting the rate constants listed in Table I into eq 1–3.

relatively low but also because "hydrolysis of acetals and ketals derived from ordinary carbonyl compounds and aliphatic alcohols is not subject to general-acid catalysis".[9] If the overall rate-determining step is the formation of oxonium cation,[9] which is preceded by the protonation of one of the ethylene glycol oxygens, then an electron-donating substituent is expected to accelerate the hydrolysis. Such a substituent effect on the hydrolysis of C_9 derivatives of **2** and, ultimately, the biological activity of the derivatives as prodrugs is currently under investigation.

From the data presented in Figure 2, a second-order rate constant for the specific acid catalyzed hydrolysis of **1** was obtained: 2.24×10^{-2} s^{-1} M^{-1}.

The values of k_2 were obtained from eq 2 through iterative computations. Approximated values were first generated from a data point where $d[2]/dt = 0$ (i.e., $k_1[1] = k_2[2]$). These values were used in calculating the concentrations of **2**, which were then compared with experimental data. More refined values of k_2 were then computed. In general, no more than three iterations were required to yield a curve which fits the data most satisfactorily. The values of k_2 thus obtained at three pH's are listed in Table I together with the k_2 values estimated from the literature.[3f] Finally, the concentration of **3** was calculated by substituting the values of k_1 and k_2 into eq 3. Concentration profiles for all three species are illustrated in Figure 3 for the data obtained at pH 4.53.

As pH decreases, k_1 increases more rapidly than k_2 (Table I) to result in virtually no accumulation of **3** during the conversion of **1** to **2**. For instance, at pH 2 and 25 °C, the half-life for the hydrolysis of **1** to **2** is in the order of 60 min, whereas that for **2** to **3** is in the order of 14 days. Even at pH 3.5, by the time 70% of **1** disappears, less than 5% of **3** is produced.

When 5.62 mg of **1**, equivalent to 5.0 mg of **2**, in 0.1 M THAM buffer of pH 8.5 was orally administered to a female rhesus monkey (*Macaca mulatta*) on the 100th day of pregnancy, uterine motility increased significantly; uterine contractions, measured using an open-ended catheter placed in the aminiotic cavity, occurred in burst, each burst consisting of four or five contractions. The frequency of burst was comparable with the frequency of contraction observed prior to treatment, approximately every 2 min. The amplitude of contractions was approximately 7 mmHg both before and after treatment. The administration of 16.87 mg of **1**, equivalent to 15 mg of **2**, caused a further increase in the frequency of contractions and a slight increase in contraction amplitude. These results, when compared with those observed when **2** was administered, appeared to indicate that **1** and **2** are bioequivalent within the experimental errors.

In conclusion the kinetic data on the acid-catalyzed hydrolysis of **1** to **2** and the preliminary data on the biological response after oral administration of **1** appeared to support that **1** readily cleaves to **2** in the stomach without much loss due to the conversion of **2** to **3**.

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