Kinetics and Mechanism of Hydrolysis of 1-(2′-Acetoxybenzoyl)-2-deoxy-α-D-glucopyranose, a Novel Aspirin Prodrug

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Abstract □ The formation rate of aspirin from the prodrug was determined as a function of the pH, temperature, and dielectric constant of the solvent spectrophotometrically and was confirmed by high-pressure liquid chromatography. Aspirin formation was first order with respect to the prodrug and zero order with respect to the hydroxide-ion concentrations. The hydrolysis rate was independent of buffer concentration but very sensitive to the dielectric constant of the solvents. The half-life for the formation of aspirin at 37° was 7 min. The activation energy for the hydrolysis was 23.7 kcal/mole. The results suggest that the hydrolysis of the prodrug to aspirin proceeds by an S_N1-type mechanism.

Keyphrases □ 1-(2′-Acetoxybenzoyl)-2-deoxy-α-D-glucopyranose—aspirin prodrug, kinetics and mechanism of hydrolysis, effect of pH, temperature, and dielectric constant □ Aspirin prodrug—kinetics and mechanism of hydrolysis, effect of pH, temperature, and dielectric constant □ Prodrugs, aspirin—kinetics and mechanism of hydrolysis, effect of pH, temperature, and dielectric constant □ Hydrolysis—aspirin prodrug, kinetics and mechanism, effect of pH, temperature, and dielectric constant

Oral administration of aspirin induces gastric irritation and bleeding (1–3) because of local irritation of the gastric mucosal membrane by the very acidic aspirin particles. One approach to minimizing this side effect is to mask the acidic carboxyl group of aspirin reversibly *via* a prodrug. Upon administration, the neutral derivative dissolves first and then hydrolyzes, generating aspirin in solution.

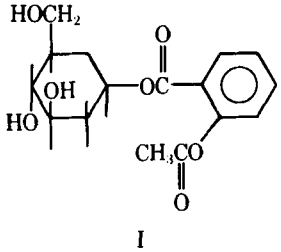
Classical esterification of the carboxyl group of aspirin results in nonirritating but insoluble species, which do not revert to aspirin. For example, the methyl ester of aspirin hydrolyzes to methyl salicylate (4). Another neutral aspirin derivative, aspirin anhydride, was absorbed slowly and incompletely (5) and was highly immunogenic (6).

An acylal-type derivative can lead to a prodrug that cleaves rapidly to aspirin (7). This report describes the hydrolysis kinetics of 1-(2′-acetoxybenzoyl)-2-deoxy-α-D-glucopyranose (I).

EXPERIMENTAL

Material and Equipment—Compound I was synthesized and characterized in this laboratory[1]. Prodrug I was characterized by elemental analysis.

Anal.—Calc. for $C_{15}H_{18}O_8$: C, 55.20; H, 5.57. Found: C, 55.35; H, 5.59.



I

In addition, the ^{13}C-NMR, ^1H-NMR, and IR spectra are consistent with the structure of I. All reagents were of high quality; aspirin USP, spectral grade dioxane, deuterium chloride, and deuterium oxide were used without further purification.

A recording spectrophotometer[2] and a high-pressure liquid chromatograph[3] with a detector[4] were used. The instrument and column conditions for high-pressure liquid chromatographic (HPLC) analysis were: column, 2.6 mm i.d. × 3 × 0.5 m long, packed with pellicular polyamide adsorbent[5]; detector, UV at 254 or 280 nm; mobile phase, 1.7% phosphoric acid in water; flow rate, 1.5 ml/min; pressure, 1000–1100 psi; and internal standard, benzoic acid.

The concentration of aspirin generated from the prodrug was calculated from the peak height using a standard solution of aspirin. The pH of the solutions was measured using a research pH meter, and the temperature was maintained constant at the desired value using a circulating water bath.

Kinetic Measurements—The I hydrolysis rate was determined by the spectrophotometric method. Solutions of about 5 mg of com-

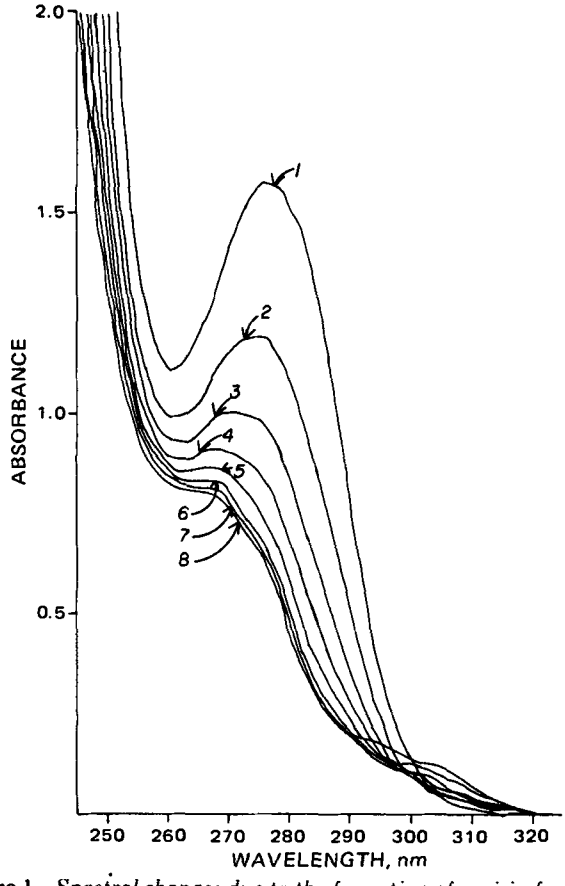


Figure 1—*Spectral changes due to the formation of aspirin from the prodrug as a function of time at 28° and pH 6. Key (elapse time from t_0 in minutes): 1, 0; 2, 20; 3, 40; 4, 60; 5, 80; 6, 100; 7, 120; and 8, 140.*

[1] A. Hussain, J. Truelove, and H. Kostenbauder, unpublished work.

[2] Cary model 15.
[3] Elmer model 1220.
[4] DuPont model 835.
[5] Pellamidon, Catalog No. A008, ReeveAngel Co., Clifton, N.J.

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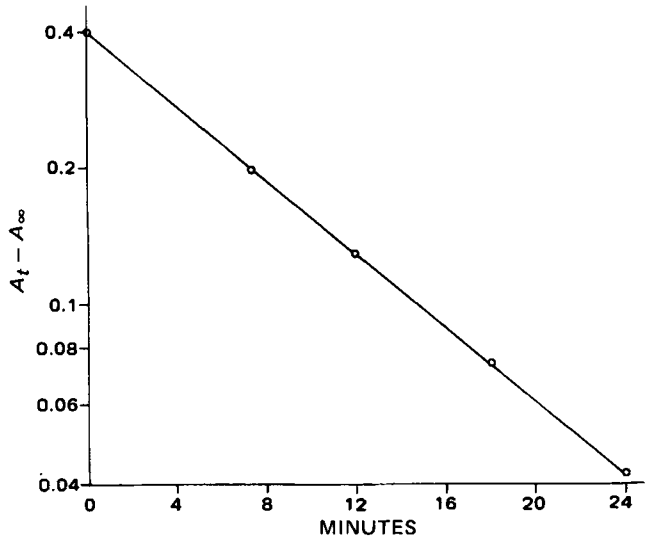


Figure 2—*First-order plot for the hydrolysis of the prodrug to aspirin at pH 6 and 28°.*

pounds/ml were prepared and maintained at 25, 27, 30, or 37° in a circulating water bath. At appropriate time intervals, 200 μl of the reaction mixture was added to 3 ml of buffer at the desired pH in a 1-cm path length spectrophotometer cell; after inverting the cell several times to ensure a uniform mixture, the absorbance was observed at 285 nm against a blank consisting of the buffer only.

Observed first-order rate constants were obtained by linear regression analysis of log $[A_t - A_\infty] = k/2.303(t) + b$[6]. In some cases, where the absorbance value at time infinity (seven half-lives) was not available due to conversion of aspirin to salicylic acid, rate constants were obtained by linear regression analysis of log $[\Delta A/\Delta t] = k/2.303(t) + b$.

To verify that the decreasing absorbances observed in the spectrophotometric method were indeed the result of generation of aspirin from the prodrug, an HPLC experiment was performed.

A solution of the prodrug at the desired pH was maintained at 22°, and 4-μl aliquots were injected periodically onto the column. The aspirin appearance rate was then estimated from the slope of the line of points generated by plotting log aspirin [peak height ($t = \infty$) − peak height (t)] *versus* time.

Buffers employed were hydrochloric acid (pH 1–2), citrate (pH 3), acetate (pH 4–6), and phosphate (pH 7–9). Ionic strength was adjusted with potassium chloride (usually to 0.1). In addition, a buffer dilution experiment was carried out with acetate buffers at pH 4.75 and a constant ionic strength (1 *M*) over a 10-fold concentration range.

Solvent dielectric effects were determined at 37° with solutions of dioxane in acetate buffer (pH 4.75) at concentrations of 0, 10, 20, and 25% (v/v) dioxane. The dielectric constants of the mixtures were taken to be those of Akerlof and Short (8). Solvent deuterium isotope effects were determined at 25° and pD values of 5.2 and 1.35 utilizing acetate–deuterium chloride and deuterium chloride buffers, respectively.

All pH measurements were made with a research pH meter with a glass

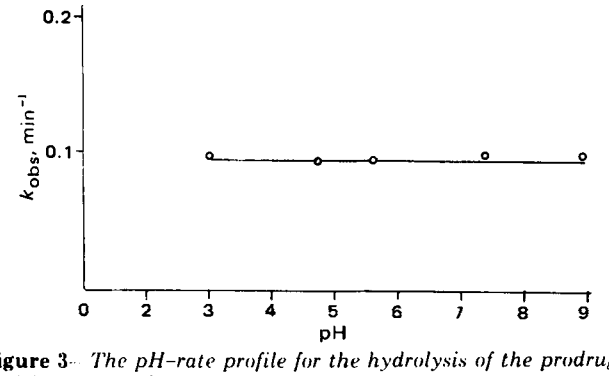


Figure 3—*The pH–rate profile for the hydrolysis of the prodrug to aspirin at 37° and μ = 0.1.*

[6] Wang program 1000-2-ST3.

Table I—Half-Lives of Hydrolysis of I to Aspirin as a Function of pH at 37°

pH	$t_{1/2}$[a], min
3	7
4.6	7.1
6.4	7.0
8	7.02
9	7.0

[a] Each value is the average of three determinations.

electrode[7]. The glass electrode gives the correct pH reading in dioxane–water mixtures. For deuterium oxide solutions, the relationship pD = pH meter reading +0.4 was employed (9).

RESULTS

The hydrolysis rate of the prodrug to aspirin was followed spectrophotometrically at 285 nm. Figure 1 shows the change in absorbance as a function of time at pH 6 and 28°. Figure 2 shows a first-order plot of $A_t - A_\infty$ *versus* time. To obtain an independent check on the spectrophotometric data, the hydrolysis rate also was determined by HPLC by measuring aspirin appearance as a function of time.

A semilog plot of aspirin [peak height ($t = \infty$) − peak height (t)] *versus* time resulted in a straight line. Furthermore, at the completion of the reaction, 1 mole of aspirin was formed from every mole of the prodrug. Figure 3 and Table I show the effect of pH on the prodrug hydrolysis rate. Figure 4 shows the effect of the dielectric constant of the solvent on the I hydrolysis rate.

The effect of temperature also was determined at pH 5.6 (Fig. 5). The activation energy was 23.7 kcal/mole, and the entropy of activation was +5 eu. The solvent deuterium isotope effect, determined at 25° and pD 5.2, gave a ratio of 0.86 for k_{D_2O}/k_{H_2O}[8].

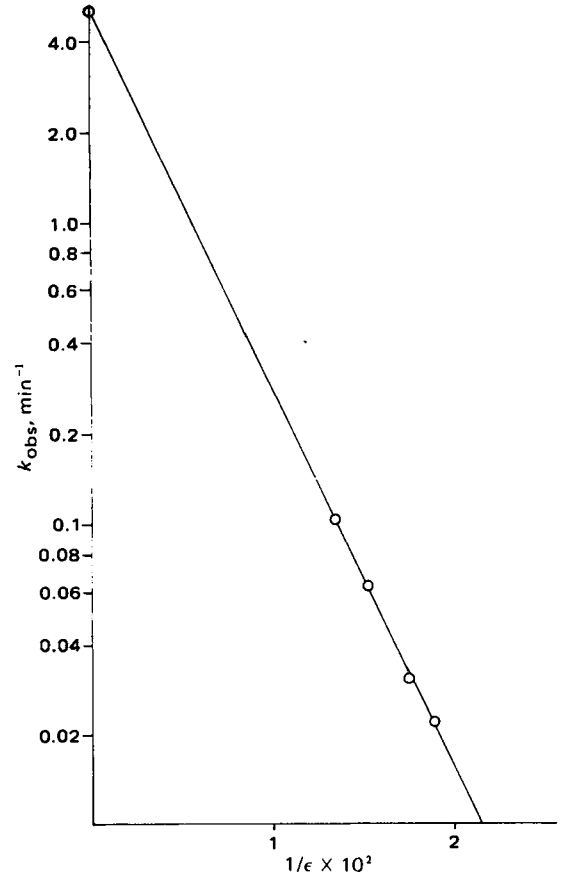


Figure 4—*Plot of the log of the observed rate of hydrolysis of the prodrug at pH 4.75, 37°, and μ = 1 as a function of 1/ε of the solvent.*

[7] Beckman model 1019.

[8] The observed first-order rates of hydrolysis of I at 25° in acetate-buffered deuterium chloride and water are 0.0170 and 0.0198 min^{-1}, respectively.

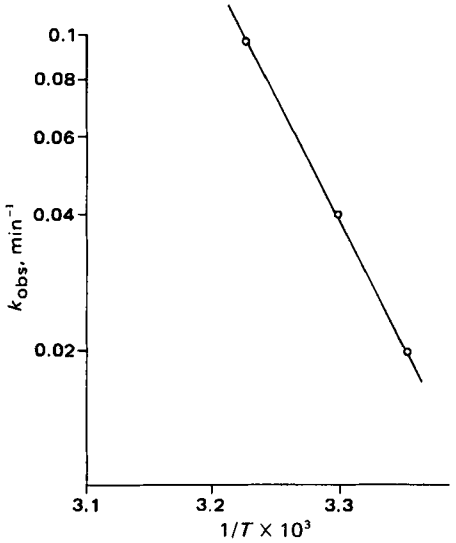
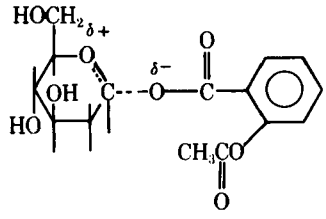


Figure 5—*Arrhenius plot for the hydrolysis of the prodrug at pH 5.6.*

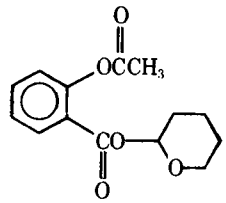
Buffer concentrations of up to 1 *M* acetate buffer at pH 5.4 did not affect the hydrolysis rate.

DISCUSSION

The hydrolysis of the prodrug to aspirin followed first-order kinetics. The most likely mechanism occurring in the pH 3–9 range is an uncatalyzed unimolecular decomposition. The absence of buffer catalysis and the slightly positive entropy of activation make catalysis by water unlikely (10). Additionally, the pronounced effect of the solvent dielectric constant on the hydrolysis rate provides evidence for increasing separation of changes in the transition state. Finally, the absence of a significant solvent deuterium isotope effect provides evidence that specific acid catalysis is not occurring at this pH range.



II



III

Based on these data, it is possible that I hydrolysis proceeds *via* the formation of the charged intermediate (II), which then undergoes simple CO bond cleavage, generating aspirin. In the same pH range, the hydrolysis rate of the corresponding pyran derivative (III) proceeds by a factor of 60 times faster than prodrug I[1].

The data presented show that the transient blocking of the acidic carboxylic group of aspirin by formation of deoxyglucose acylal-linked derivatives can result in a prodrug that regenerates aspirin at an acceptable rate independent of pH. Such a compound could potentially reduce the GI liability of aspirin by presenting a neutral molecule to the gastric membrane. Plans are underway to test this concept *in vivo*.

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Kinetics and Mechanism of Reaction of *m*-Nitrobenzhydrazide and Other Hydrazines with Acetic Acid

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Abstract □ A comprehensive kinetic study was conducted of the reactions of *m*-nitrobenzhydrazide and some other hydrazines with acetic acid. The overall reaction rate for all of the compounds studied followed pseudo-first-order kinetics. The temperature dependence of the *m*-nitrobenzhydrazide degradation reaction was determined. The reaction rate dependence on the acetic acid concentration was found to be close to first order. High-pressure liquid chromatography was used extensively in identifying and measuring the appearance or disappearance rates of *m*-nitrobenzhydrazide degradation products in acetous solution. With *m*-nitrobenzhydrazide, the major degradation products were *N,N'*-bis(*m*-nitrobenzoyl)hydrazine, *N*-acetyl-*N'*-*m*-nitrobenzoylhydrazine, diacetylhydrazine, and hydrazine. The concentration profiles of these products in solution suggested a complex mechanism by which hydrazides react with acetic acid. All eight rate constants at 61° in the suggested mechanism were calculated by an approximation method based on experimental data. The findings in the present study indicate that acetic acid is to be avoided as a solvent for hydrazine derivatives.

Keyphrases □ *m*-Nitrobenzhydrazide—kinetics and mechanism of reaction with acetic acid □ Hydrazine derivatives—kinetics and mechanism of reaction with acetic acid □ Kinetics—reactions of hydrazines with acetic acid

Hydrazides and other hydrazine derivatives constitute a large and important class of organic compounds and have long been used in the chemical and pharmaceutical fields. In analytical chemistry, they are used as good quantitative carbonyl reagents. Although hydrazides are weaker bases than other hydrazine derivatives and, therefore, possess

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