International Journal of Pharmaceutics, 6 (1980) 1–9
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Research Papers

ACID-CATALYZED HYDROLYSIS OF FOSFOMYCIN AND ITS IMPLICATION IN ORAL ABSORPTION OF THE DRUG

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(Received February 7th, 1980)
(Modified version received April 24th, 1980)
(Accepted April 29th, 1980)

SUMMARY

The kinetics of hydrolytic opening of the epoxide ring in fosfomycin were studied in aqueous solutions at 37°C over the pH range 0.25–4.0. The rate of hydrolysis showed a first-order dependency on the parent drug concentration and its variation with pH could be accounted for in terms of specific acid-catalyzed reactions of fosfomycin-free acid and the monoanionic species. Half-lives for the hydrolysis were 27, 37 and 133 min at pH 1.0, 1.2 and 2.0, respectively. Data are presented suggesting that the poor and variable bioavailability reported for fosfomycin upon oral administration can be attributed, at least in part, to hydrolytic degradation of the antibiotic in the stomach.

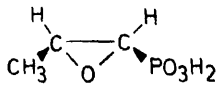
INTRODUCTION

Fosfomycin (cis-(3-methyloxiranyl)-phosphonic acid) is a relatively new antibiotic isolated from fermentation broths of several species of Streptomyces (Hendlin et al., 1969) or obtained by chemical synthesis (Christensen et al., 1969; Glamkowski et al., 1970). The antibiotic activity of the compound has been demonstrated to arise from inhibition of bacterial cell wall synthesis through an irreversible reaction with a pyruvyl transferase involved in the first step of cell wall synthesis (Kahan et al., 1974). The compound appears to have a low order of toxicity and it is used in therapy or subject to clinical trials in several countries.

Studies of the pharmacokinetics of fosfomycin have shown that the drug is poorly absorbed in man following oral administration (in the form of its calcium salt) (Foltz et al., 1970; Gallego et al., 1974; Shimizu, 1977; Cardórniga et al., 1977). Only approximately 30–40% of the dose given is absorbed and moreover, large variations in bioavailability were observed among different subjects (Shimizu, 1977). Fosfomycin or its calcium salt are highly soluble in acidic aqueous solutions (Shimizu, 1977) and slow

dissolution from the peroral preparations seems not to be a potential source for the incomplete absorption.

The presence of an epoxy group in the fosfomycin molecule makes it susceptible to hydrolysis, especially in the acidic environment of the stomach. Epoxides are known to undergo acid-catalyzed hydrolysis (Parker and Isaacs, 1959) and therefore, a possible explanation of the incomplete and variable absorption pattern of fosfomycin could be acid-catalyzed degradation in the stomach. To assess this possibility, the hydrolysis kinetics of fosfomycin have been determined in acidic aqueous solutions and under conditions similar to those found during oral absorption, and are the subject of this paper.



Fosfomycin

MATERIALS AND METHODS

Apparatus

The optical rotation experiments were performed with a Perkin-Elmer 141 Polarimeter, using a 10-cm cuvette and mercury light at 365 nm. The polarimeter cuvette was thermostatted at 37°C by circulating water. Readings of pH were carried out on a Radiometer Type PHM 26 meter at the temperature of study.

Chemicals and reagents

Samples of disodium fosfomycin with a purity of better than 98% were kindly provided by Dumex A/S, Copenhagen. Sodium metaperiodate, arsene trioxide, potassium iodide and all other chemicals used were of analytical grade.

A 0.01 M periodate solution was prepared by dissolving 2.14 g of sodium metaperiodate in 1000 ml of water, and 0.01 N sodium arsenite solution by dissolving 4.95 g of arsene trioxide in 50 ml of 2 M sodium hydroxide and 950 ml of water, and diluting this solution 10 times with water. A 0.2 M phthalate buffer of pH 6.5 was prepared by dissolving 41 g of potassium biphthalate in water, adjusting pH with 10 M sodium hydroxide and adding water to make 1000 ml.

Determination of the 1,2-diol hydrolysis product

The 1,2-diol product formed upon hydrolytic opening of the epoxide ring of fosfomycin was determined by the common periodate oxidation method for diols (cf. Dryhurst, 1970). A 1000 μl aliquot portion of the reaction mixture initially containing about 4 mg ml^{-1} of fosfomycin was added to 50 ml of the phthalate buffer solution, pH 6.5, followed by the addition of 5.00 ml of 0.01 M periodate solution. The solution was set aside for 20 min in the dark and then 10 ml of a 40% aqueous potassium iodide solution were added. After standing for 2 min the liberated iodine arising from the excess of periodate was titrated with 0.01 N arsenite using starch as indicator. The end-point was taken as the volume of arsenite required to give a colourless solution stable for one

minute. A blank was run in a similar way. The difference between the blank and sample arsenite titers is equivalent to the periodate consumed and hence to the concentration of the 1,2-diol present. Control experiments showed that fosfomycin did not interfere in the assay.

Kinetic measurements

All kinetic experiments were carried out in aqueous buffer solutions (hydrochloric acid, perchloric acid, phosphate or acetate) at 37.0 ± 0.1°C. The reaction rates were determined by following the formation of the hydrolysis product using the periodate assay or by monitoring the decrease in optical rotation at 365 nm resulting from opening of the epoxide ring (cf. Fig. 1). In the case of the first method, reaction solutions containing about 4 mg ml^{-1} of disodium fosfomycin were kept at 37°C in a water-bath and at various intervals, samples of 1000 µl were taken and analyzed for 1,2-diol compound as described above. For the polarimetric measurements reaction solutions with an initial concentration of disodium fosfomycin of about 20 mg ml^{-1} were placed in the thermostatted polarimeter cell and the decrease in rotation was followed as a function of time. Readings of pH of the reaction solutions were done at the end of the hydrolysis to ensure that no pH changes had occurred.

Determination of ionization constants of fosfomycin

The apparent ionization constants of fosfomycin were determined by potentiometric titration at 37°C of 0.02 M disodium fosfomycin solutions using 1 M hydrochloric acid as titrant. Calculation of pK_a-values was performed as described by Albert and Serjeant (1971).

RESULTS AND DISCUSSION

Kinetics of fosfomycin degradation in acidic aqueous solution

The rates of degradation of fosfomycin in aqueous solution were measured at 37°C over the pH range 0.25–4.0. At constant pH and temperature the hydrolysis displayed strict first-order kinetics over more than 4 half-lives. When the direct polarimetric method was used, pseudo-first-order rate constants (k_{obs}) were calculated from the slope of linear plots of $\log(\alpha_t - \alpha_\infty)$ against time, where α_t and α_∞ are the optical rotations of the reaction solutions at time t and at infinite time, respectively. A typical first-order plot of optical rotation data is given in Fig. 1. When the progress of hydrolysis was followed by the titrimetric periodate assay for a glycol degradation product pseudo-first-order rate constants were derived from linear plots of $\log(A_\infty - A_t)$ against time, where A_∞ and A_t represent ml of consumed arsenite titers at infinite time and at time t, respectively. As demonstrated by the rate data in Fig. 2 the values of k_{obs} derived using these methods were in favourable agreement. The relative standard errors for the observed rate constants were estimated to be in the range 2–4%.

The rates of hydrolysis were found to be unaffected by variation (0.05–0.2 M) in the concentration of the phosphate and acetate buffers used to maintain constant pH within the range pH 2.2–4.0 (cf. Table 1). The results in Table 1 also show that changes in the ionic strength of the reaction solutions effected through addition of sodium perchlorate

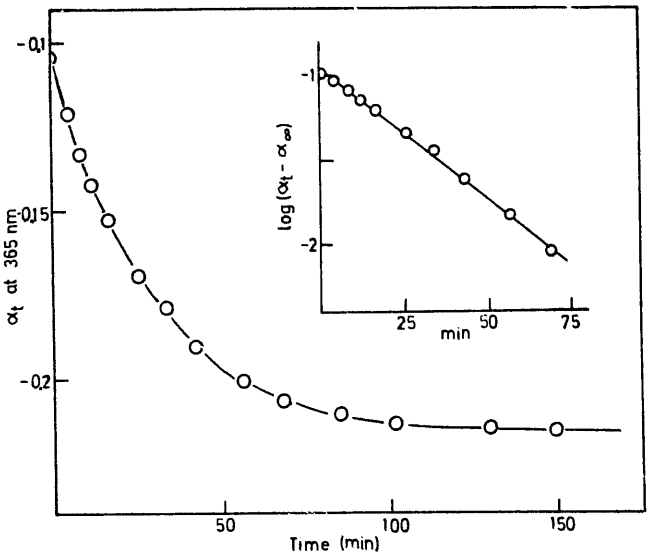


Fig. 1. Decrease in optical rotation at 365 nm with time of an aqueous solution of disodium fosfomycin (18 mg ml^{-1}) of pH 0.85 at 37°C. The inset is a first-order plot of the rotation–time data.

are without influence on the hydrolysis rate. However, addition of chloride ions gave rise to a small increase in the rate constants at pH 1.15. This effect is most likely due to a nucleophilic attack of chloride ion on the protonated epoxy group to produce a chlorohydrin product as has been described for the reaction of chloride ions with various other epoxides (e.g. Addy and Parker, 1963, 1965) including arene oxides (Whalen and Ross, 1976). The perchlorate ion has an extremely low nucleophilic activity and is known to be

TABLE 1
Pseudo-first-order rate constants for the degradation of fosfomycin in aqueous solutions at 37°C

Medium	pH	k_{obs} (min^{-1})
Perchloric acid	0.25	0.10
Hydrochloric acid	0.85	3.5×10^{-2}
Hydrochloric acid with 1 M sodium chloride	1.15	2.5×10^{-2}
Hydrochloric acid with 0.5 M sodium chloride	1.15	2.1×10^{-2}
Hydrochloric acid with 1 M sodium perchlorate	1.15	1.8×10^{-2}
Hydrochloric acid	1.15	1.8×10^{-2}
Hydrochloric acid	1.69	9.9×10^{-3}
0.2 M phosphate buffer	2.88	8.9×10^{-4}
0.1 M phosphate buffer	2.88	8.8×10^{-4}
0.2 M acetate buffer	4.00	7.0×10^{-5}
0.1 M acetate buffer	4.00	7.2×10^{-5}
Simulated gastric fluid USP XIX	1.20	2.0×10^{-2}

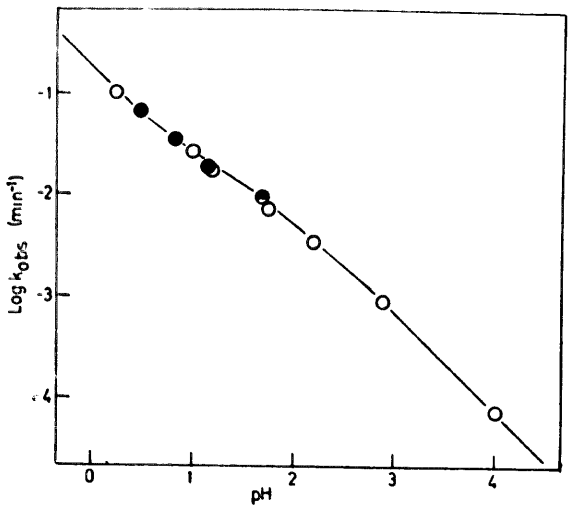


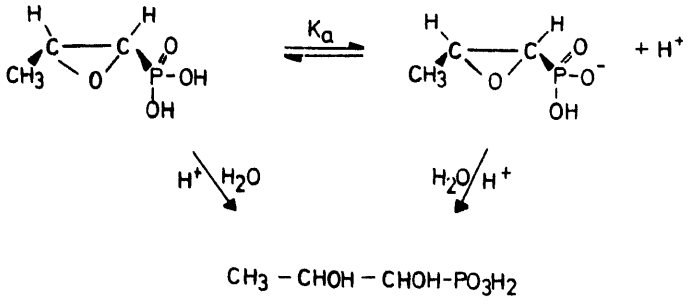
Fig. 2. The pH–rate profile for the hydrolysis of fosfomycin in aqueous solution at 37°C. The points refer to experimental values obtained using the polarimetric (•) or the periodate method (○) while the curve is calculated on basis of Eqn. 1 and the values of K_a, k_H and k'_H given in the text.

unable to attack epoxides in acidic aqueous solutions (e.g. Pritchard and Siddiqui, 1972; Whalen and Ross, 1976).

The presence of pepsin in the acidic solutions had an insignificant effect on the degradation rate as can be seen by comparing the k_{obs}-values obtained with simulated gastric fluid USP XIX and with a hydrochloric acid solution of similar pH (Table 1).

The pH-dependence of the rate of hydrolysis of fosfomycin at 37°C is shown in Fig. 2 in which the logarithm of the observed pseudo-first-order rate constants have been plotted against pH. In the ranges pH < 0.5 and pH > 3 the pH–rate profile shows two linear segments with slopes of unity while a partial plateauing is observed between these pH ranges.

In the pH range studied (0.25–4.0) fosfomycin can exist in free acid form and as monoanion; the pK_a values were determined to be 1.7 and 6.7 (at 37°C). The shape of the pH–rate profile suggests that the free acid and the monoanionic forms both undergo specific acid-catalyzed hydrolysis with the ionic species being more reactive (Scheme 1).



Scheme 1

According to Scheme 1 the kinetic data are described by Eqn. 1:

$$k_{obs} = k_H a_H \frac{a_H}{a_H + K_a} + k'_H a_H \frac{K_a}{a_H + K_a} \quad (1)$$

where a_H refers to the hydrogen ion activity, $a_H/(a_H + K_a)$ and $K_a/(a_H + K_a)$ are the fractions of total fosfomycin in the free acid and monoanionic form, respectively, and K_a is the apparent first ionization constant of fosfomycin acid (equal to $10^{-1.7}$). The second-order rate constants k_H and k'_H for the acid-catalyzed hydrolysis of acid and monoanion forms, respectively, were derived in the following manner. Dividing Eqn. 1 by a_H and substituting $a_H/(a_H + K_a)$ with $(1 - K_a/(a_H + K_a))$ gives Eqn. 2:

$$k_{obs}/a_H = k_H + (k'_H - k_H)\frac{K_a}{a_H + K_a} \quad (2)$$

According to this equation a plot of k_{obs}/a_H against $K_a/(a_H + K_a)$ should give a straight line. As seen from Fig. 3, treatment of the rate data in this way resulted in a linear plot. From the intercepts of the plot the following values of k_H and k'_H were derived:

$k_H = 0.17\ M^{-1}\ min^{-1}$; and $k'_H = 0.70\ M^{-1}\ min^{-1}$.

The solid line drawn in Fig. 2 has been calculated from Eqn. 1 and these values for the rate constants and the good agreement between the calculated and experimental data demonstrate that the rate expression of Eqn. 1 is consistent with the observed kinetics of hydrolysis.

The only previously reported data on the stability of fosfomycin in acidic aqueous solutions were found in the absorption study by Shimizu (1977). Using a microbiological assay this investigator measured the residual activity of fosfomycin calcium salt in hy-

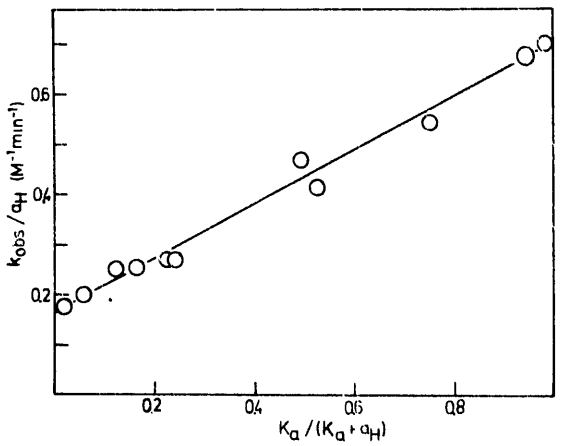
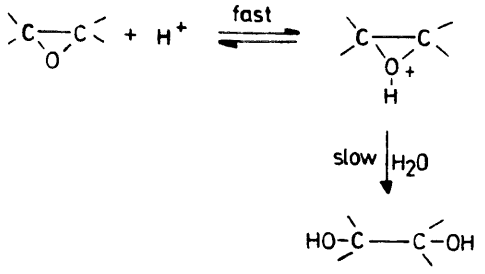


Fig. 3. Derivation of k_H and k'_H by plotting the rate data according to Eqn. 2.

drochloric acid solutions incubated for various times at 37°C. From the reported data pseudo-first-order rate constants at pH 1.2 and 1.6 can be calculated to be 0.02 and 0.006 min^{-1}, respectively. As seen from Table 1 these values are close to those found in the present study using other methods for following the degradation process.

Regarding the mechanism of the acid-catalyzed hydrolysis of fosfomycin it seems likely that the A-2 mechanism depicted in Scheme 2 is involved as has been proposed to



Scheme 2

be the case for primary and secondary alipathic epoxides (Parker and Isaacs, 1959; Pritchard and Siddiqui, 1973; Carr and Stevenson, 1973; Wohl, 1974). This mechanism is characterized by initial protonation of the epoxide moiety in a fast reversible step followed by reaction of the protonated epoxide with a water molecule in a rate-limiting step. The polar effect of substituents on the initial equilibrium step has been shown to be dominant over that on the second, rate-determining step in that electron-withdrawing substituents retard the acid-catalyzed ring opening (Addy and Parker, 1965). The greater reactivity of fosfomycin monoanion over the free acid form, as seen from the values of k_H and k'_H, can be rationalized on this basis since the phosphonic acid group is more electronegative than its monoanion.

Bioavailability aspects

The results presented show that fosfomycin is rapidly hydrolyzed in acid solutions. On the basis of Eqn. 1 pseudo-first-order rate constants and the corresponding half-lives for hydrolysis were calculated at various pH values and are shown in Table 2. The hydrolysis rate is highly dependent upon pH and an important factor in the peroral bioavailability of fosfomycin would be the gastric acidity and also the gastric emptying rate. These parameters vary widely among and within individuals and are influenced by numerous factors such as the emotional state and food ingestion (Cooke, 1975; Mayersohn, 1979). Normally, gastric pH is within the range 1–2 and a gastric emptying half-time of about 50 min can be taken as a reasonable mean value (Griffith et al., 1968; Heading et al., 1973; Digenis et al., 1977). It is apparent from these figures and the rate data in Table 2 that the poor bioavailability reported for fosfomycin upon oral administration may be attributed, at least in part, to hydrolytic degradation of the compound in the stomach. Also, the variability in oral fosfomycin observed among individuals (Shimizu, 1977) may arise from different extents of hydrolysis due to variations in gastric pH and gastric emptying rate. If the assumptions are made that the absorption of fosfomycin primarily takes place outside the stomach as has been claimed by Gallego et al. (1974) and the gastric emptying process is associated with a first-order rate constant of 0.014 min^{-1} (derived from a $t_{1/2}$

TABLE 2
Pseudo-first-order rate constants and half-lives for the hydrolysis of fosfomycin in aqueous solution at 37°C

pH	k^a (min^{-1})	$t_{1/2}$ (min)
0.0	0.17	4.1
1.0	0.026	26.7
1.2	0.019	36.5
1.7	0.0087	79.7
2.0	0.0052	133
2.5	0.0020	347

[a] The figures were calculated from Eqn. 1 and the experimentally determined values of K_a, k_H and k'_H.

value of 50 min), it can be calculated from the data in Table 2 and using the equations for two parallel first-order processes that 35% of an oral dose of fosfomycin would leave the stomach intact at a gastric pH of 1.0 and 73% if the gastric pH is 2.0. In the absorption study made by Shimizu (1977) the gastric juice collected from the subjects used in the investigation was found to have a pH of 1.

It has been described (Shimizu, 1977) that when given orally together with sodium bicarbonate fosfomycin is absorbed to a somewhat greater extent. This effect can be due to an increase in the gastric pH, resulting in a decreased rate of hydrolysis.

Finally, it is of interest to note that the rates of hydrolysis of fosfomycin in the pH range 1–2 are of the same order of magnitude as the rates for digoxin hydrolysis under similar conditions (Sternson and Shaffer, 1978; Khalil and El-Masry, 1978). These investigators have suggested that the variability in oral digoxin absorption observed among and within individuals may arise from variations in gastric pH, which influence the extent to which hydrolysis occurs.

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