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GI Absorption of β-Lactam Antibiotics III: Kinetic Evidence for *In Situ* Absorption of Ionized Species of Monobasic Penicillins and Cefazolin from the Rat Small Intestine and Structure–Absorption Rate Relationships

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Abstract □ Absorption rates of monobasic β-lactam antibiotics were measured as a function of lumen solution pH between 4 and 9 by utilizing the rat intestinal recirculating method *in situ*. Between pH 6.5 and 9, the absorption rate constants of ionized antibiotics were almost identical; but, at pH 4, the unionized species were highly absorbed, depending on their lipophilicity through the GI membrane lipoidal barrier. The structure-absorption rate relationship was established with the unstirred layer model.

Keyphrases □ Absorption, GI—various monobasic penicillins and cefazolin, effect of ionization, pH, structure–activity relationships □ Penicillins, various—GI absorption, effect of ionization, structure–activity relationships □ Cefazolin—GI absorption, effect of ionization, structure–activity relationships □ Antibiotics—penicillins and cefazolin, GI absorption, effect of ionization, structure–activity relationships

The GI absorption rate of a monobasic penicillin in rats deviated significantly from the pH-partition hypothesis (1). This shift was interpreted successfully by the absorption mechanism of penicillins through the aqueous diffusion layer of the lumen side of the GI membrane, which is restricted by the lipophilicity of the undissociated species (1).

In *in vitro* experiments utilizing an isolated gut technique, the transport of phenoxypenicillin derivatives exhibited a saturable process, but additional study did not produce evidence of active transport (2). A similar *in vitro* study (3) provided evidence for the passive transport of β-lactam antibiotics at pH 7.4. Perrier and Gibaldi (4) attributed the larger absorption of dicloxacillin than of penicillin G and ampicillin from the *in situ* rat intestinal loop to the larger lipophilicity of dicloxacillin. A good correlation was found (5) between the rate of *in situ* intestinal cephalexin and cefazolin absorption in rats and their *in vitro* release rate from liposomes.

Recent studies in this laboratory on the *in situ* absorption kinetics of low lipophilic and completely ionized amphoteric β-lactam antibiotics, such as amoxicillin (6), cyclacillin (7), and cephradine (7), indicated that their intestinal absorption is governed by simple diffusion fol-

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lowing first-order kinetics at a high dose and is favored by some forms of a carrier-mediated transport process following Michaelis–Menten kinetics at low doses. However, the kinetics and mechanism for the transport of ionized species of monobasic β-lactam antibiotics across the intestinal mucosa are not clearly understood.

In the present absorption study, attention was paid to lumen solution pH, and simultaneous absorption and degradation were assessed. Discussion by others (4) on the absorption of ionized penicillin species seems to harbor some questions because of the pH change and β-lactam ring degradation, both of which may occur during absorption experiments, especially with the *in situ* method. In this report, kinetic evidence is presented for the *in situ* intestinal absorption of ionized forms of monobasic penicillins and cefazolin. Furthermore, based on the unstirred layer theory, which is applicable to the absorption of undissociated β-lactam antibiotic molecules (1), the relationship between the absorption rate of antibiotics and their oil–water partition coefficients has been established.

EXPERIMENTAL

Materials—The following β-lactam antibiotics were used as supplied: penicillin V potassium[1] (1490 units/mg), phenethicillin potassium[1] (1444 units/mg), propicillin potassium[2] (993 μg/mg), oxacillin sodium[3] (840 μg/mg), cloxacillin sodium[1] (907 μg/mg), dicloxacillin sodium[1] (900 μg/mg), floxacillin sodium[4] (893 μg/mg), and cefazolin sodium[4] (966 μg/mg). Solutions of penicilloic acids of propicillin and dicloxacillin were prepared according to the method of Schwartz and Delduce (8).

All other chemicals were reagent grade and were used without further purification, except for imidazole which was purified by double recrystallization from benzene followed by thorough washing with ether.

***In Situ* Intestinal Absorption Procedure**—Male albino Wistar rats, 225 ± 25 g, were fasted over 20 hr prior to the experiments, but water was given freely. The rats were anesthetized with urethan, 1.3 g/kg ip.

The recirculating perfusion procedure for studying *in situ* intestinal absorption was essentially the same as that described previously (1, 9), except for the use of the upper 30-cm portion of the small intestine. The bile duct was ligated in all experiments. The small intestine was washed with 50 ml of perfusion solution, and a 5-ml solution of a penicillin or of a penicilloic acid (5 mg/ml, unless otherwise stated) was recirculatingly perfused at 2 ml/min. The drug solution was prepared with isotonic buffer (10), and the lumen solution pH was maintained at the desired pH with a pH-stat[5] (1, 9) during the absorption experiments.

The perfusion periods were 1 hr at pH 4.0 and 2 hr at other pH values. At the end of the absorption experiment, the recirculating drug solution of about 9 ml and a fresh isotonic buffer perfused to wash the intestine were collected into a 50-ml volumetric flask; the required amount of the same buffer was added to obtain 50 ml. To obtain a final sample, the solution was passed through a 0.45-μm filter[6] to remove any solid materials. The initial sample was prepared by diluting 5 ml of the initial drug solution (5 mg/ml) with the isotonic buffer to obtain 50 ml.

Analytical Procedures—The antibiotic concentration was determined by three methods: UV spectrophotometry (11), high-pressure liquid chromatography (HPLC), and spectrofluorometry (12). Due to the interference by unknown materials with UV absorbance in the sample solution of penicillins having an isoxazole group (oxacillin, cloxacillin, dicloxacillin, and floxacillin) (4), these samples were analyzed only by HPLC.

HPLC—The chromatograph[7] was equipped with a variable-wavelength UV detector[8] set at 210 or 254 nm. A reversed-phase column[9], prepacked with octadecylsilane chemically bonded on totally porous silica gel, was used for the determination of unchanged β-lactam antibiotics. The eluent was 10–30% (v/v) aqueous acetonitrile containing 0.01 *M* ammonium acetate. For the determination of penicilloic acids, an anion-exchange column[10] was utilized with 0.02 *M* KH_2PO_4 adjusted to pH 5 as the eluent; a 100-μl sample was injected through a loop injector on flow. Peak heights were used for quantification. A standard solution of the corresponding penicilloic acids was prepared according to the procedure described by Schwartz and Delduce (8).

Spectrofluorometry—Spectrofluorometry was used in the analysis of relatively lower propicillin concentrations and of the sample from the absorption experiment with penicilloic acid. This analytical method was based on the formation of a fluorescent Schiff base resulting from the reaction between 5-dimethylaminonaphthalene-1-sulfonylhydrazine and penilloaldehyde (12), which can be produced from the reaction of penicilloic acid and mercuric chloride at pH 2.5. This method has the advantage of simultaneous determination of the intact penicillin and its product, penicilloic acid (12). The detailed procedure was described elsewhere (12)[11].

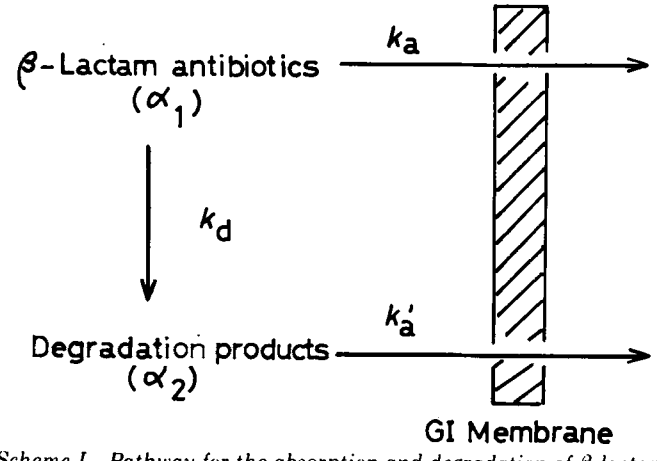
RESULTS AND DISCUSSION

Simultaneous Absorption and Degradation—Table I shows the percentage of remaining penicillins and the percentage of penicilloic acids that appeared after 2 hr in the *in situ* intestinal recirculating absorption experiments for propicillin, dicloxacillin, penicillin V, and cefazolin. Samples were analyzed by HPLC for all penicillins and cefazolin and by spectrofluorometry for propicillin and penicilloic acids. There were significant degradations in the β-lactam moiety of penicillin derivatives. Cefazolin degradation was negligible during the experiments since the corresponding product peak, which was observed in *in vitro* degradation for a sufficiently long period under the same condition, was not detected.

Possible absorption of penicilloic acids was examined under similar conditions. Both penicilloic acids of propicillin and dicloxacillin were absorbed to the same extent (16.1 ± 6.8%) at the end of 2 hr at pH 7.4. From these results, the overall pathway of β-lactam antibiotics in the intestinal lumen solution can be represented as in Scheme I.

As seen in Table I, the widespread change of the initial propicillin concentration between 100 and 5000 μg/ml produced an almost constant disappearance of 23% and an appearance of 10% of the penicilloic acid at pH 7.4. A similar result was reported (2) for penicillin V disappearance in the 0.6–160-μg/ml range from rat intestinal lumen solution at pH 7.4. These findings indicate that all rate processes in Scheme I follow apparent first-order kinetics, at least for propicillin and penicillin V. If one assumes that a similar kinetic process applies to other β-lactam antibiotics according to Scheme I, the ratios of a remaining antibiotic (α_1) and an apparent penicilloic acid (α_2) at time t to the initial antibiotic dose would be expressed by:

$$\alpha_1 = e^{-(k_a + k_d)t} \qquad \text{(Eq. 1)}$$



Scheme I—Pathway for the absorption and degradation of β-lactam antibiotics. (For the meaning of α_i, see text.)

[1] Meiji Seika Kaisha, Tokyo, Japan.
[2] Takeda Chemical Industries, Osaka, Japan.
[3] Banyu Pharmaceutical Co., Tokyo, Japan.
[4] Fujisawa Pharmaceutical Co., Osaka, Japan.
[5] pH-Stat titrator assembly consisting of TTT2 titrator and ABU12b autoburette, Radiometer, Copenhagen, Denmark.
[6] Sartorius-Membranfilter GmbH, Göttingen, Germany.
[7] Model FLC-A700, Japan Spectroscopic Co., Tokyo, Japan.
[8] Model UVIDEC-100, Japan Spectroscopic Co., Tokyo, Japan.
[9] μBondapak-C_{18}, 30 cm × 4 mm i.d., Waters Associate, Milford, Mass.
[10] Zipax-SAX, 50 cm × 2.1 mm i.d., DuPont Instrument, Wilmington, Del.
[11] In the previous paper (12), the concentration of 5-dimethylaminonaphthalene-1-sulfonylhydrazine reagent was erroneously referred to as 10%. The correct concentration is 0.1%.

Table I—Summary of the Data Determined for Penicillin, Penicilloic Acid, and Cefazolin in the *In Situ* Absorption Experiments through the Small Intestine of the Rat

β-Lactam Antibiotic	pH	Dose, μg/ml	Remaining Antibiotic[a], % (100 × α_1)	Appearance of Penicilloic Acid[a], % (100 × α_2)	Rate Constant[b], 10^3 min^{-1}	
					k_a or k'_a	k_d
Propicillin	6.5	5,000	69.1	7.1	2.30	0.78
	7.4	100	69.1	15.6	1.38	1.70
	7.4	100	70.0	8.8	2.02	0.96
	7.4	100	84.9	7.6	0.61	0.75
	7.4	500	80.2	5.7	1.26	0.58
	7.4	500	84.8	2.8	1.10	0.28
	7.4	500	84.9	9.5	0.43	0.94
	7.4	1,000	67.4	15.2	1.61	1.68
	7.4	1,000	87.2	4.8	0.67	0.47
	7.4	5,000	74.0	8.6	1.60	0.91
	7.4	5,000	73.9	15.0	0.93	1.59
	7.4	5,000	66.9	18.0	1.35	2.00
	7.4	10,000	87.0	0.8	1.08	0.08
	7.4	10,000	84.9	7.5	0.62	0.74
	7.4	10,000	98.6	0.3	0.09	0.03
	7.4	10,000	86.5	5.8	0.64	0.57
	8.0	5,000	79.1	3.6	1.59	0.37
	8.5	5,000	82.8	3.8	1.19	0.38
	8.5	5,000	80.9	5.4	1.22	0.55
	9.0	5,000	69.6	19.4	0.91	2.11
	9.0	5,000	79.0	7.0	1.25	0.72
Dicloxacillin	6.5	5,000	74.5	15.1	0.86	1.59
	7.4	5,000	79.8	7.9	1.08	0.80
	7.4	5,000	69.6	4.9	2.49	0.53
	8.0	5,000	73.3	7.5	1.79	0.80
	8.5	5,000	75.2	7.1	1.63	0.74
	8.5	5,000	84.0	7.0	0.76	0.69
	8.5	5,000	76.9	9.0	1.26	0.93
	9.0	5,000	79.2	12.8	0.63	1.31
Penicillin V	6.5	5,000	77.7	7.0	1.38	0.72
	7.4	5,000	82.8	10.4	0.53	1.04
	8.5	5,000	78.5	8.2	1.18	0.84
	8.5	5,000	81.2	8.2	0.91	0.83
	9.0	5,000	69.6	18.1	1.05	1.97
Penicilloic acid of propicillin	7.4	5,000	88.1	—	1.06	—
	7.4	5,000	89.9	—	0.90	—
	7.4	5,000	84.0	—	1.45	—
Penicilloic acid of dicloxacillin	7.4	5,000	83.7	—	1.48	—
	7.4	5,000	74.7	—	2.43	—
Cefazolin	7.4	5,000	89.4	—	0.93	—
	7.4	5,000	89.2	—	0.95	—
	7.4	5,000	92.4	—	0.66	—

[a] Determined at the end of 2-hr experiments. [b] Calculated from Eqs. 4 and 5 by use of $\alpha_0 = 0.839$, which is the average of values determined for penicilloic acids of propicillin and dicloxacillin.

$$\alpha_2 = \frac{k_d}{(k_a + k_d) - k'_a}\left[e^{-k'_a t} - e^{-(k_a + k_d)t}\right] \qquad \text{(Eq. 2)}$$

where k_a and k'_a represent the apparent first-order absorption rate constants of a penicillin and its penicilloic acid, respectively, and k_d is the apparent first-order penicillin degradation rate constant. The absorption rate constant, k'_a, can be calculated from:

$$\alpha_0 = e^{-k'_a t} \qquad \text{(Eq. 3)}$$

where α_0 is the ratio of the remaining penicilloic acid to the initial dose, which is determined under the same conditions in a separate absorption experiment for penicilloic acid. In the present kinetic treatment, the value of k'_a or α_0 was regarded as independent of lumen solution pH and of the kind of penicilloic acids, being equal to 1.46×10^{-3} min^{-1} or 0.839 of the mean value of k'_a or α_0 as determined for the penicilloic acids of dicloxacillin and propicillin. By using the data of α_1, α_2, and α_0, the penicillin absorption rate constant, k_a, and degradation rate constant, k_d, could be calculated from Eqs. 1–3 using:

$$k_a = -\frac{1}{t}\left(\ln \alpha_1 + \frac{\alpha_2}{\alpha_0 - \alpha_1} \ln \alpha_0/\alpha_1\right) \qquad \text{(Eq. 4)}$$

$$k_d = \frac{\alpha_2}{t(\alpha_0 - \alpha_1)} \ln \alpha_0/\alpha_1 \qquad \text{(Eq. 5)}$$

The raw data of α_1, α_2, and α_0 and k_a and k_d of penicillins and cefazolin determined under various conditions are summarized in Table I, and their mean values are listed in Table II.

The rate constants of chemical degradation under similar conditions were known to be almost independent of the kinds of penicillins but dependent on lumen solution pH. They were evaluated, based on previous kinetic data (13), as 1.0×10^{-4}, 1.3×10^{-4}, and 6.0×10^{-4} min^{-1} in isotonic buffer of pH 6.5, 7.4, and 9.0, respectively. When comparing the degradation rate constants of penicillins under *in situ* and *in vitro* conditions, it must be emphasized that, at pH 9.0, both were in a similar range. At a neutral pH of 6.5–7.4, however, the degradation rate constants *in situ* were ~6–10 times greater than those *in vitro*. These results suggest that in the intestinal lumen and/or at the intestinal mucosa, β-lactam cleavage other than chemical reaction takes place in an apparently first-order kinetic fashion under physiological pH conditions.

The present kinetic study provided evidence for the significant absorption of ionized penicillins and cefazolin and also for the absorption of penicilloic acids. If the intestinal absorption of ionized β-lactam antibiotics is the result of transport through the lipoidal barrier, as was verified (1) for the undissociated forms, significant differences in the extent of absorption may be expected among penicillins. In spite of the 10-fold difference at maximum in the oil–water partition coefficient (14) of the ionized forms of penicillin V, propicillin, and dicloxacillin, the ionized species of these penicillins and cefazolin were absorbed to an almost identical extent (Table II).

This result suggests that intestinal absorption of ionized β-lactam antibiotics below the concentration of 5 mg/ml follows apparent first-order kinetics such as, for example, simple diffusion through water channels in the membrane and that it is almost insensitive to the lipophilicity of transport molecules. However, due to the slight decrease of propicillin absorption at 10 mg/ml (Table II), the possibility cannot be

Table II—Mean Values Describing the Kinetic Behavior of β-Lactam Antibiotics and Penicilloic Acid in Rat Lumen Solution and Dose Effect on the Absorption of Propicillin

Antibiotic	Dose, μg/ml	Total Disappearance[a], % ± SD	Appearance of Penicilloic Acid[a] % ± SD	Rate Constant[b], 10^3 min^{-1} ± SD: Absorption, k_a or k'_a	Rate Constant[b], 10^3 min^{-1} ± SD: Degradation, k_d	Number of Experiments
Propicillin	100[c]	25.3 ± 8.9	10.7 ± 4.3	1.34 ± 0.71	1.14 ± 0.50	3
	500[c]	16.7 ± 2.7	6.0 ± 3.4	0.93 ± 0.44	0.60 ± 0.33	3
	1,000[c]	22.7	10.0	1.14	1.08	2
	5,000[c]	28.4 ± 4.1	13.9 ± 4.8	1.29 ± 0.34	1.50 ± 0.55	3
	5,000[d]	25.0 ± 5.7	9.8 ± 6.1	1.37 ± 0.42	1.05 ± 0.68	9
	10,000[c]	10.8 ± 6.3	3.6 ± 3.6	0.61 ± 0.41	0.36 ± 0.35	4
Dicloxacillin	5,000[d]	23.4 ± 4.4	8.9 ± 3.4	1.31 ± 0.63	0.92 ± 0.35	8
Penicillin V	5,000[d]	22.0 ± 5.1	10.4 ± 4.5	1.01 ± 0.32	1.08 ± 0.51	5
Penicilloic[e] acid	5,000[c]	16.1 ± 6.8	—	1.46 ± 0.59	—	5
Cefazolin	5,000[c]	9.7 ± 1.8	—	0.85 ± 0.16	—	3

[a] Observed values at the end of 2-hr experiments. [b] Calculated value by Eqs. 4 and 5. [c] Averaged value at pH 7.4. [d] Averaged value between pH 6.5 and 9. [e] Penicilloic acids of propicillin and dicloxacillin prepared by Schwartz and Delduce (8).

excluded that a specialized mechanism of intestinal carrier-mediated transport of the ionized species exists, as was proposed for the intestinal absorption of completely ionized and highly hydrophilic aminopenicillins and aminocephalosporins (6, 7).

Effect of pH on Intestinal Absorption and Structure–Absorption Rate Relationships—Figure 1 shows the plots of *in situ* absorption rate constants, k_a, of penicillins *versus* perfusion solution pH, which was maintained with a pH-stat (1, 9). Absorption rate constants at pH 4.0 were calculated, assuming the degradation to be negligible in comparison to absorption, while those at other pH values were derived from Eq. 4.

Between pH 6.5 and 9, the penicillin absorption was independent of the lumen solution pH, with average rate constants, k_a, of 1.27×10^{-3} min^{-1}; it was almost identical among penicillin molecules (Table II). Below pH 6, the rate constants increased significantly with a decrease of the lumen solution pH depending on the lipophilicity of the undissociated species. Therefore, k_a can be expressed as:

$$k_a = k_u + k_i \qquad \text{(Eq. 6)}$$

where k_u and k_i are the apparent first-order absorption rate constants for unionized and ionized species of penicillins, respectively. A previous study (1) verified that k_u can be expressed by Eq. 7, which is derived from the kinetic model of membrane permeation across the lipoidal barrier of undissociated penicillin species transported through the aqueous diffusion layer with thickness L_I adjacent to the surface of the lipoidal membrane with thickness L_{II}:

$$k_u = \frac{SD_{aq}}{VL_I}\left(\frac{f_u}{T + f_u}\right) \qquad \text{(Eq. 7)}$$

where:

$$T = \frac{L_{II}D_{aq}}{L_I D_{lip} Pl_u R} \qquad \text{(Eq. 8)}$$

$$f_u = \frac{a_{H^+}}{K_a + a_{H^+}} \qquad \text{(Eq. 9)}$$

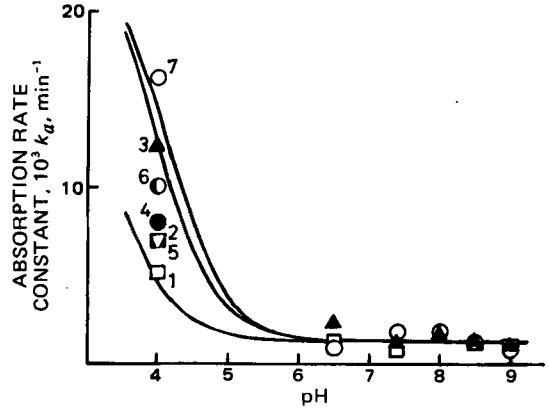


Figure 1—*Plots of* in situ *rat intestinal absorption rate constants,* k_a, *for penicillins* versus *pH of the perfusion solution at 37°. The compounds are numbered as in Table III. The points are experimental values, and the solid lines were generated from Eqs. 6, 12, and 14;* k_i = 1.27×10^{-3} min^{-1} *for Compounds 1, 3, and 7.*

where V is the volume of the recirculating drug solution; D_{aq} and D_{lip} are the diffusion coefficients of the drug in water and lipid, respectively; Pl_u is the partition coefficient of the undissociated drug between the lipid membrane and aqueous solution; f_u is the fraction of undissociated species; a_{H^+} is the hydrogen-ion activity of the lumen solution; K_a is the penicillin dissociation constant; and R is the ratio of the true interfacial area to the geometrical area, S, of the membrane.

Parameters V, L_I, L_{II}, S, and R can be regarded as constant since V and L_I depend only on experimental conditions and the others depend on the membrane. By using the relationship of Eq. 10 (10, 15) for a diffusion coefficient, D, and of Eq. 11 (10) for a partition coefficient, Pl_u, Eq. 7 can be simplified to Eq. 12:

$$D = \beta_1(\sqrt{MW})^{-1} \qquad \text{(Eq. 10)}$$

$$Pl_u = \beta_2 P_u \qquad \text{(Eq. 11)}$$

$$k_u = k_a - k_i = \frac{1}{a\sqrt{MW}}\left(\frac{f_u P_u}{b + f_u P_u}\right) \qquad \text{(Eq. 12)}$$

where MW and P_u represent the molecular weight and the partition coefficient between two immiscible liquids of the undissociated species of the transport antibiotics, respectively, and β_1 and β_2 are proportionality constants.

The rearrangement of Eq. 12 leads to:

$$(k_u\sqrt{MW})^{-1} = ab(f_u P_u)^{-1} + a \qquad \text{(Eq. 13)}$$

To verify Eq. 13, the intestinal absorption of several penicillins at pH 4.0 was examined (Table III and Fig. 2). The P_u values in the octanol–water system and the K_a values used for the calculation were determined previously (14). The plots of $1/(k_u\sqrt{MW})$ *versus* $1/(f_u P_u)$ according to Eq. 13 gave a good linear relationship (Fig. 2), and the following equation was obtained:

$$(k_u\sqrt{MW})^{-1} = 70.0(f_u P_u)^{-1} + 1.95 \qquad \text{(Eq. 14)}$$

The solid line in Fig. 1 was calculated for dicloxacillin, propicillin, and penicillin V from Eqs. 6, 12, and 14 and was in fair agreement with ex-

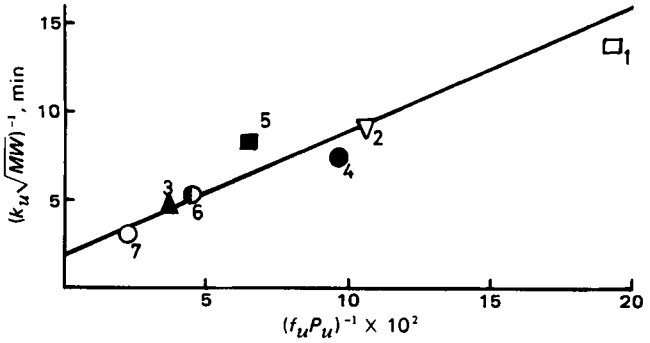


Figure 2—*Plots of* $(k_u\sqrt{MW})^{-1}$ *against* $(f_u P_u)^{-1}$ *for penicillins in* in situ *perfusion experiments at pH 4. The compounds are numbered as in Table III.*

Table III—Absorption Rate Constants at pH 4.0 and Related Parameters for Penicillins

Number	Penicillin	Molecular Weight (MW)[a]	pKa[b]	log P_u^b	k_a, 10^3 min^{-1}
1	Penicillin V	350.4	2.79	1.95	5.22
2	Phenethicillin	364.4	2.80	2.20	7.08
3	Propicillin	378.4	2.76	2.70	12.48
4	Oxacillin	401.4	2.73	2.31	8.05
5	Cloxacillin	435.9	2.78	2.43	7.05
6	Floxacillin	453.9	2.76	2.61	10.15
7	Dicloxacillin	470.3	2.76	2.91	16.25

[a] As free acid. [b] P_u is the partition coefficient of the undissociated penicillins in the octanol–water system. All data were at 37° and taken from Ref. 14.

perimental values. This finding indicates that the absorption of monobasic penicillins, excluding amphoteric ones such as amoxicillin and cyclacillin (6, 7), follows the common mechanisms of: (*a*) the lipoidal membrane transport of the undissociated species permeating the aqueous diffusion layer barrier and (*b*) the apparent first-order transport of the ionized species permeating some forms of the barrier almost insensitive to antibiotic lipophilicity.

A previous study (1) revealed that, below pH 6, the intestinal absorption rate of propicillin is about 100 times faster than the gastric absorption rate. This significant difference is undoubtedly due to the relative surface area in the alimentary tract. Naturally, other monobasic β-lactam antibiotics can be expected to exhibit absorption behavior similar to propicillin. The present and previous results (1, 9) indicate that a small amount of these orally ingested antibiotics may be absorbed in the stomach while almost all absorption takes place in the duodenum, where the fluid pH is relatively lower, and that absorption occurs *via* passive diffusion of the undissociated species, largely dependent on their lipophilicity. A small amount may be also absorbed by ionic transport during the slow transit through the intestine. Poor absorbability (16) of orally ingested cephalosporins such as cefazolin, the P_u of which is about one-thirtieth lower than that of penicillin V (17), may be due to the considerable reduction of k_u values effective both in the stomach and upper duodenum.

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Pyridones as Potential Antitumor Agents

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Abstract □ Based on the finding that 3-acetoxy-2-pyridone had reproducible activity against murine P-388 lymphocytic leukemia, derivatives in this series were synthesized and evaluated to determine structural parameters important for activity. Of the 32 compounds tested, 10 were active. At least two oxygen-containing functional groups are required for P-388 activity, and the 2,3-isomeric arrangement provides the greatest activity. Carbamate or acyloxy groups in the 3-position produced the most active 2-pyridones.

Keyphrases □ Pyridone derivatives—antineoplastic activity, structure–activity relationships, mice □ Antineoplastic agents, potential—pyridone derivatives, structure–activity relationships, mice □ Structure–activity relationships—pyridone derivatives, antineoplastic activity, mice

During an investigation of hydroxypyridine derivatives, it was discovered that 3-acetoxy-2-pyridone (I) possessed reproducible activity against P-388 leukemia in mice. There are other scattered reports of pyridone antitumor activity. A nucleoside presently undergoing clinical evaluation, which can be classed as a pyridone, is 3-deazauridine (1, 2). Mimosine, a naturally occurring 4-pyridone, was reported as active against Walker 256 carcinosarcoma (3) and B16 melanotic melanoma (4). Other pyridones studied possess less antitumor activity (5–8). Based on the antitumor properties of I, a study was undertaken to determine the structural parameters important for activity.

RESULTS AND DISCUSSION

Compounds I–XXXII were evaluated. 3-Hydroxy-2-pyridone (II) was reacted with various acid chlorides, anhydrides, and isocyanates to produce 3-substituted and 1,3-disubstituted 2-pyridones. 2-Pyridone (VII) was oxidized to 5-hydroxy-2-pyridone (IV) by the Elbs persulfate reaction. UV and other spectral data were consistent with those expected for 2-pyridones (9, 10). The properties of these compounds are shown in Table I.

Antitumor activity was evaluated in the P-388 lymphocytic leukemia system using standard protocols (11) of the National Cancer Institute. Multiple biological tests were carried out with each compound using a dose response on the QD 1–9 treatment schedule. Physiological saline was the vehicle. Drugs were administered intraperitoneally to mice with

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