[Chem. Pharm. Bull. 29(7)1986—1997(1981)]

Biopharmaceutical Study on the Oral and Rectal Administrations of Enamine Prodrugs of Amino Acid-like β-Lactam Antibiotics in Rabbits[1)]

Teruo Murakami,* Hiromu Tamauchi, Masaru Yamazaki, Kazuyoshi Kubo, Akira Kamada,[2a)] and Noboru Yata[2b)]

Faculty of Pharmaceutical Sciences, Osaka University,[2a)] 133-1 Yamada-Kami, Suita, Osaka, 565, Japan, and Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine,[2b)] 1-2-3 Kasumi, Hiroshima, 734, Japan

(Received September 4, 1980)

Enamine derivatives of ampicillin, amoxicillin and cephalexin were prepared by reacting the drugs with ethyl acetoacetate under mild conditions. The enamine structures of these compounds were determined by measurements of the nuclear magnetic resonance (NMR) spectra. The compounds were easily hydrolyzed *in vitro* in aqueous solution and the rate accelerated as the pH was lowered. The determination of *Rf* and rate of migration of the enamine derivatives showed that the enamine derivatives were more lipophilic than the corresponding parent drugs. The bioavailabilities of these prodrugs were studied following oral and rectal administrations in rabbits. The bioavailabilities of the parent antibiotics were not improved by the oral administration of the corresponding enamine prodrugs. However, the bioavailabilities of ampicillin and amoxicillin were markedly improved by rectal administration of the corresponding enamine prodrugs. The improved bioavailabilities exceeded those of the parent drugs following oral administration in rabbits. The enamine prodrug of ampicillin was found to promote the rectal absorption of ampicillin upon coadministration of these two drugs. Thus, enamine derivatives of amino acid-like β-lactam antibiotics appear to be interesting candidates for possible clinical use as prodrugs for rectal administration.

Keywords——ampicillin, amoxicillin and cephalexin; enamine prodrugs of amino acid-like β-lactam antibiotics; rectal administration of antibiotics; improvement of bioavailability; absorption promotion of ampicillin

Ampicillin has been clinically administered by the oral route but its intestinal absorption is considered to be rather unsatisfactory. The poor absorbability is assumed to be attributable to the amphoteric character of the drug at physiological pH.[3)] Purich *et al.*[4)] studied the pH-partition properties of amino acid-like β-lactam antibiotics and found that the minimum partitioning occurred at the iso-electric pH region of the antibiotics.

Many attempts have been made to improve the partitioning properties of ampicillin at physiological pH for the purpose of enhancement of its intestinal absorptivity. In attempts to improve the oral bioavailability of ampicillin, many prodrugs have been prepared by employing masking agents of the carboxyl group as well as of the amino group.[5)]

Hetacillin[6–8)] has been prepared as an amino group-masked prodrug of ampicillin.

Another example of an amino group-protected compound is the enamine derivative; such derivatives have been obtained as intermediates in antibiotics synthesis.[9,10)] However, none of the intermediates was isolated or tested for therapeutic usefulness.

Among some studies on enamine prodrugs as potential medical agents, Caldwell *et al.*[11)] prepared enamine derivatives of phenyl propanolamine and tested their pharmacological activities in rats. They considered enamine derivatives to be potentially useful prodrug derivatives.

Among many β-dicarbonyl compounds applicable to the synthesis of enamine prodrugs of amino acid-like β-lactam antibiotics, ethyl acetoacetate (EtAA) is considered to be one of the best compounds in view of its low toxicity[12)] and it is an officially approved food additive as a flavoring agent for chewing gum, ice cream and fruit juice in Japan. Thus, in the present study, EtAA was used as a masking agent for the amino group of amino acid-like β-lactam

NII-Electronic Library Service

antibiotics.

The present report concerns the synthesis of enamine prodrugs of ampicillin, amoxicillin and cephalexin and describes biopharmaceutical studies on oral and rectal administrations of the derivatives to rabbits and dogs.

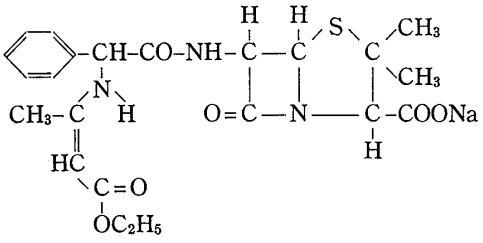
Experimental

Materials——Samples of sodium ampicillin (ABPC Na), amoxicillin trihydrate (AMPC·$3H_2O$), cephalexin monohydrate (CEX·H_2O), talampicillin hydrochloride (TAPC·HCl) and potassium hetacillin (IPABPC K) were obtained from commercial sources through the courtesy of Sawai Pharmaceutical Co., Ltd. and Kyoto Pharmaceutical Industries., Ltd., and used without further purification. All other reagents and solvents were commercial products of reagent grade and were used without further purification.

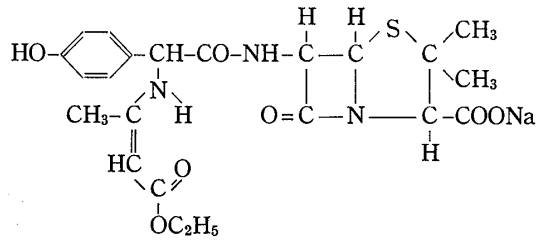
Synthesis of Enamine Prodrugs——Reaction of Sodium Ampicillin with EtAA: EtAA (1.1 mol) was added to a suspension of ABPC Na (1.0 mol) in a sufficient amount of isopropanol and the whole was stirred for 3 h at 25°C. The solution was filtered through a sintered glass filter (G-3). The filtrate was poured into an excess of ethyl ether to give a precipitate. The precipitate was repeatedly crystallized from dioxane by the addition of ethyl ether to give N-(1-methyl-2-ethoxycarbonylvinyl) ampicillin sodium (ABPC EtAA Na) in a yield of 75%.

Other enamine derivatives were similarly prepared, with minor modification of the procedure if necessary, from the sodium salts of antibiotics and EtAA. Sodium salts of antibiotics were prepared by reacting the antibiotics with sodium 2-ethylhexanoate in a mixture of isopropanol and dimethyl formamide and precipitating the product by the addition of benzene. The chemical structures of enamine derivatives synthesized and related prodrugs are listed in Table I with their abbreviations.

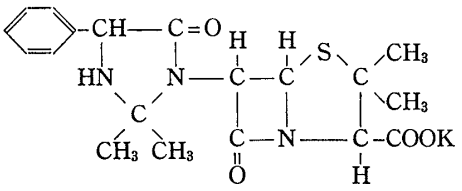
TABLE I. Chemical Structures of β-Lactam Antibiotic Prodrugs and Their Abbreviations



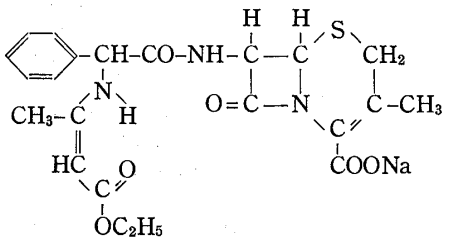
ABPC EtAA Na



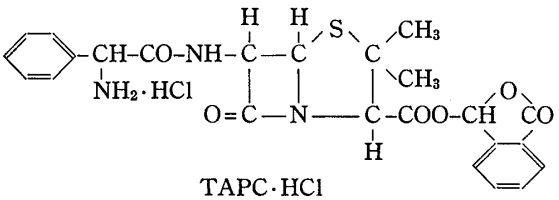
AMPC EtAA Na



IPABPC K



CEX EtAA Na



TAPC·HCl

Animal Studies——Five to ten fasted male albino rabbits, weighing 2.5—3.0 kg, and three male beagles, weighing 12—15 kg, were used for each experiment. The doses of antibiotics were fixed at 15 mg/kg for ABPC Na and AMPC·$3H_2O$ and 50 mg/kg for CEX·H_2O. The doses of the enamine derivatives were the same in terms of the amount of the corresponding parent drug.

Intravenous Administration: To determine the apparent bioavailability, a saline solution (0.3 ml/kg) of each parent antibiotic was administered to a marginal ear vein of a rabbit. At 0, 1, 3, 5, 7, 9, 12, 15, 20, 30, 40, 50 and 60 min after administration, 0.2 ml blood samples were collected from a marginal vein of the other ear with heparinized syringes. They were kept in a refrigerator at 4°C until assay.

NII-Electronic Library Service

Oral Administration: Exact amounts of a drug were weighed and filled in a No. 1 hard gelatin capsule (Nippon Elanco Co., Ltd., Osaka, Japan). A rabbit was fixed in the crouching position and the mouth was forced open with a wooden rod having a hole at the center. A capsule attached to the lower end of a rubber stomach tube with rubber adhesive tape was inserted into the stomach and 20 ml of water were forced into the stomach through the tube to detach the capsule.

For the study of enteric coated capsules in dogs, capsules were coated with methyl acrylate–methacrylic acid–methyl methacrylate copolymer (MPH-06, Tanabe Pharmaceutical Co., Ltd., Osaka, Japan). Hard gelatin capsules containing a designated amount of drug were repeatedly immersed in a 10 w/v% ethanol solution of the polymer and dried at room temperature until 10 mg of the polymer was coated on each capsule. These capsules did not dissolve in pH 3.5 buffer at 37°C for more than 2 h but easily dissolved in a pH 6.5 buffer at 37°C within 10 min. A dog was manually forced to open its mouth, then a capsule was placed at the entrance of the esophagus. The dog was forced to close its mouth, made to lean backwards and rubbed down to allow the capsule to be pushed into the stomach. Twenty ml of water was administered from a feeding bottle to ensure that the capsule was swallowed. Blood samples were collected from the jugular vein at 0, 15, 30, 45, 60, 80, 100, 120, 150, 180, 210 and 240 min after the administration.

Rectal Administration: Rectal suspensions of drugs were prepared at 40°C immediately prior to the experiments by dispersing a drug into fused rectal base manually with a mortar and pestle. The concentration of drug in the rectal suspension was fixed at 10%. Four bases were used: Witepsol H-15 (Dynamit Novel Chemicals, Troisdorf-Oberlar, West Germany), Migryol 812 (Dynamit Novel Chemicals, Troisdorf-Oberlar, West Germany), a mixture of equal amounts of liquid paraffin (J. P. IX) and white petrolatum (J. P. IX) and a mixture of equal amounts of white petrolatum and squalane (Nikko Chemicals Co., Ltd., Tokyo, Japan).

Following an overnight fast, rabbits were fixed in the crouching position. An exact amount of rectal suspension kept at 37°C was taken into a disposable 1 ml syringe. After insertion of the tip of the barrel about 7 mm into the rectum, the suspension was forced into the rectum, and the anus was kept clamped with a plastic clip during the experimental period of 4 h. The doses of each drug were the same as those used in oral administrations. At 0, 10, 20, 40, 60, 80, 100, 120, 150, 180, 210 and 240 min after the dosing, 0.2 ml blood samples were collected from a marginal ear vein.

Analytical Methods——Assay of Antibiotics: Enamine derivatives were separated from parent drugs by bioautography.

Plastic sheets, 5 × 10 cm, coated with silica gel (Silica Gel 60 F_{254}, TLC Plastic Sheets, Wako Pure Chemical Ind. Ltd., Osaka, Japan) were used. The mobile phase was ethyl acetate: acetic acid (96: 4). After development, the plastic sheet was dryed in the air, and then sprayed with 1% Na_2CO_3 solution. The sheet was placed on an agar plate containing *Sarcina lutea* ATCC 9341. After overnight incubation, the relative fractions of enamine derivative and parent drug were determined by comparison of the inhibition zone diameters on the agar plate.

By the above bioautography, 0.1 μg/ml of enamine derivative in plasma was detectable. At the dose of enamine derivatives used in the present study, no enamine fraction was detected in any of the blood samples after oral or rectal administration. This finding suggests that the enamine moiety was hydrolyzed to the parent drug in the process of absorption and/or in the blood stream. Thus, the usual microbiological disk diffusion method[13] was used for the analysis of all samples as follows; 0.2 ml of blood sample was diluted with 1 ml of distilled water. The concentrations of antibiotics in blood samples were measured with *Sarcina lutea* ATCC 9341 as the test organism and a sensitive test agar "Eiken" (E-MC10, Eiken Chemical Co. Ltd., Tokyo, Japan).

Nuclear Magnetic Resonance (NMR) Measurements: The spectra were obtained at 31°C on a 90 MHz NMR Spectrometer (model R-22, Hitachi Co. Ltd., Tokyo, Japan). Samples were dissolved in dimethyl sulfoxide-d_6 at a concentration of 5 w/w%. Tetramethylsilane was used as an internal reference. The techniques of addition of D_2O and proton decoupling were used to assign some protons.

Ultra Violet (UV) Measurements: The UV absorption spectra of ethanol solution of the enamine derivatives were obtained with a recording spectrophotometer (model UV 200, Shimadzu Seisakusho, Ltd., Kyoto, Japan).

Hydrolysis of the Enamine Moiety: A spectrophotometric method was used to determine the rates of hydrolysis of enamine derivatives to the parent drugs in aqueous solution. Enamine derivatives were dissolved in phosphate buffers of various pH values (ionic strength, 0.15; concentration, 0.5 mM). The concentration of enamine fraction in solution was measured in terms of the absorbance at the maximum absorption wavelength of each compound. The results were treated according to apparent first-order kinetics.

Determination of Values of *Rf* and Rm: A chromatographic technique was employed for estimation of the hydrophilic-lipophilic characteristics of drugs in terms of the values of *Rf* and extrapolated Rm.[14]

Glass plates, 20 × 20 cm, coated with silica gel G (Type 60, E. Merck, Darmstadt, Germany) at an average layer thickness of 0.22 mm were used. The mobile phases used for the determination of *Rf* values were acetone: acetic acid (96: 4) or ethyl acetate: acetic acid (96: 4). The plates were dried at room temperature, and the chromatograms were visualized by spraying a 1% aqueous solution of $KMnO_4$. For the determina-

NII-Electronic Library Service

tion of Rm values, glass plates coated with silica gel G were impregnated with liquid paraffin by developing the plates with a solution of liquid paraffin–ethyl ether (3: 97 w/v) in a covered developing chamber and allowing them to dry in air at room temperature. A finish line was marked on the liquid paraffin-impregnated plate about 1 cm from the top of the plate. With a 10 μl micropipette, aliquots of ethanol solutions of compounds were spotted on a line 2 cm from the bottom of the plates. Plates were developed with various mixtures of veronal buffer at pH 7.4 and acetone. The *Rf* values of compounds were obtained in terms of the concentration of acetone. The Rm values were calculated by means of the following equation.[14]

$$\mathrm{Rm} = \log(1/Rf - 1)$$

A plot of the Rm values against the concentration of acetone in the mobile phase gave a linear relationship. The theoretical Rm values corresponding to 0% acetone concentration were obtained by extrapolation. They were designated as the extrapolated Rm values.

Results and Discussion

NMR Spectra

To confirm the structures of the 1: 1 condensation products of EtAA and ABPC, AMPC and CEX, proton resonance studies were made. Assignments of protons in the products were performed by means of deuterium exchange and decoupling experiments. The NMR spectra of ABPC and ABPC EtAA in deuterated dimethyl sulfoxide with or without deuterium oxide are presented in Figs. 1 and 2, respectively. ABPC showed a fortuitous coincidence of chemical shifts for both protons in the β-lactam ring, as already reported by workers.[15] However, for ABPC EtAA, an ABX-type triplet was observed for the two protons and the addition of D_2O to the solution resulted in two doublets at 5.50 and 5.68 ppm (J=4.0 Hz). The doublet signal of the N–H proton in the enamine moiety was eliminated by the addition of D_2O, resulting in the change of the doublet signal of C–H in the glycine moiety into a singlet signal. At the same time, a singlet signal of the vinyl proton in the enamine moiety was eliminated by the addition of D_2O. From these observations, the structure of ABPC EtAA was confirmed to be the enamine derivative in dimethyl sulfoxide solution. Similarly, the EtAA condensates of AMPC Na and CEX Na were also confirmed to be the enamine derivatives. However, in the case of cyclacillin (ACPC), EtAA condensates of ACPC Na were found to be the enamine or Schiff base or a mixture of the two according to the conditions of synthesis on the basis of NMR data. Thus, no further physicochemical and biopharmaceutical studies

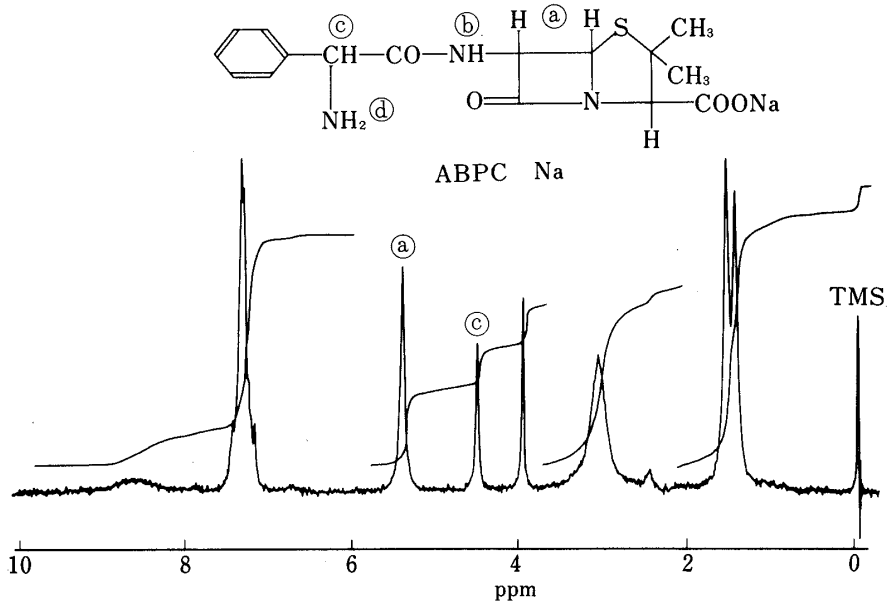


Fig. 1. NMR Spectrum of ABPC Na

The samples was dissolved in dimethyl sulfoxide-d_6.

NII-Electronic Library Service

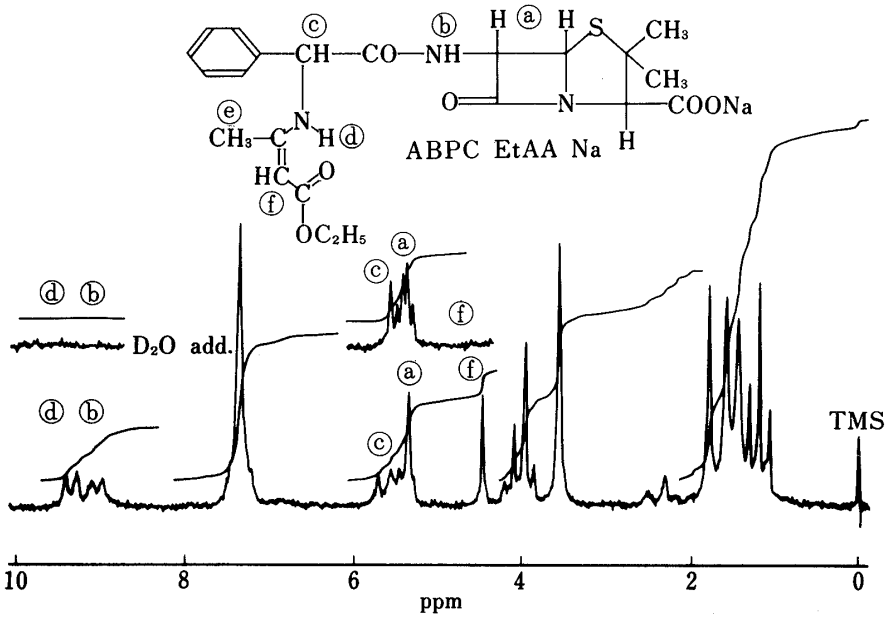
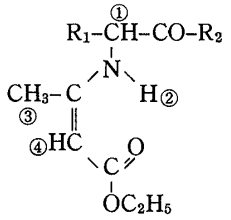


Fig. 2. NMR Spectrum of ABPC EtAA Na

The sample was dissolved in dimethyl sulfoxide-d_6 and D_2O was added.

TABLE II. Chemical Shifts of ABPC EtAA Na, AMPC EtAA Na and CEX EtAA Na



	R_1	R_2
ABPC EtAA Na:	C_6H_5	6-APA Na
AMPC EtAA Na:	C_6H_4–OH	6-APA Na
CEX EtAA Na:	C_6H_5	7-ACA Na

	① (1H)	② (1H)	③ (3H)	④ (1H)
ABPC EtAA Na	5.78(d, 10.0)	9.67(d, 10.0)	1.87(s)	4.54(s)
AMPC EtAA Na	5.55(d, 9.0)	9.16(d, 9.0)	1.76(s)	4.40(s)
CEX EtAA Na	5.50(d, 9.0)	9.53(d, 9.0)	1.76(s)	4.46(s)

① -D_2O→ singlet.
② and ④ disappeared upon D_2O addition.
Each sample was dissolved in dimethyl sulfoxide-d_6.
The letters s and d in parenthesis designate singlet and doublet peaks, respectively.
Numbers in paren thesis are the coupling constants (Hz).

were attempted because of the possible complexity arising from the tautomeric interchange of the two forms in solution. The chemical shifts of enamines studied are presented in Table II.

Physicochemical Properties

The values of *Rf* and Rm for each drug and its enamine derivative are presented in Table III.

Enamine derivatives showed high lipid affinity, suggesting good permeability through biological membranes.

The stability of enamine derivatives to hydrolysis of the enamine moiety in aqueous solution is presented in Table III in terms of pH-profiles of the apparent first-order reaction rate constants at 25 °C, activation energies at pH 7.4, and half-lives at 25 °C and pH 7.4. The enamine derivatives studied were found to be easily hydrolyzed in acidic solution by general acid–base catalysis. The hydrolysis of enamine derivatives was partially inhibited by the

NII-Electronic Library Service

Table III. Properties of Enamine Derivatives of Antibiotics

	Rf A	*Rf* B	Rm[a]	λ_{max} (nm)	pH-profile[b] log K (min^{-1})	Activation energy (kcal/mol)	$t_{0.5}$[c] (min)
ABPC	0.23	0.0	0.28				
ABPC EtAA	0.84	0.70	2.45	288	−0.773pH+3.720	10	69
AMPC	0.12	0.0	0.05				
AMPC EtAA	0.80	0.53	1.53	285	−0.796pH+3.832	12	79
CEX	0.14	0.16	0.11				
CEX EtAA	0.87	0.96	1.88	283	−0.640pH+2.582	18	98

Rf (A): acetone: acetic acid=96: 4, (B): ethyl acetate: acetic acid=96: 4.
a) The extrapolated Rm value was determined in pH 7.4 veronal buffer, with silica gel G as the solid support, and liquid paraffin as the stationary phase.
b) K; Hydrolysis rate constant of the enamine moiety in phosphate buffers of various pH's (μ=0.15) at 25°C.
c) Phosphate buffer (pH=7.4, μ=0.15) at 25°C.

addition of 1% rabbit plasma. This result suggests a possible interaction with plasma protein. However, from the results of bioautographic assay of blood samples in the animal study, no fraction of enamine prodrug was found in blood samples. This finding supports the rapid hydrolysis of the enamine moiety, and suggests possible enzymatic hydrolysis in the liver and other organs.

Antimicrobial Activities

The antimicrobial activity of each enamine derivative was measured with *Sarcina lutea* ATCC 9341 and *Escherichia coli* NIH J as test microbials following the agar plate and paper disc method. The activities of enamine derivatives were found to be weaker than those of the corresponding parent drugs at pH 9, where the hydrolysis of enamine moiety was considered to be relatively minor during the microbiological assays.

However, at pH 7, which was adopted for microbiological assay in the present study, the antimicrobial activities of enamine derivatives were found to be the same as those of the parent drugs. This finding suggests that the hydrolysis of enamine derivatives during the period of diffusion and incubation can account for the present results. These results were also supported by the finding that stable enamine derivatives of ampicillin condensed with acetylacetone or α-acetylbutyrolactone showed weak antimicrobial activity against *Sarcina lutea* at pH 9 and 7. Thus, the antimicrobial activities of enamine derivatives are considered to be negligible, and the EtAA derivatives were thus proved to be prodrugs because of the regeneration of the parent antibiotics in the body by hydrolysis of the enamine moiety.

Intravenous Administration

To determine the apparent bioavailabilities of antibiotics after oral and rectal administration of the parent drugs or their enamine derivatives, ABPC Na, AMPC Na and CEX Na were intravenously administered to rabbits at doses of 15, 15 and 50 mg/kg, respectively. The values of area under the concentration–time curve (AUC) obtained graphically are presented in Table IV.

Table IV. Pharmacokinetic Parameters of Antibiotics

Parameter	ABPC Na	AMPC Na	CEX Na
Dose, mg/kg	15.0	15.0	50.0
AUC, μg·min/m	695(48)	992(245)	2620(50)

Each number represents the mean for four rabbits.
Numbers in parenthesis are the standard errors.
Sodium amoxicillin (AMPC Na) and sodium cephalexin (CEX Na) were prepared as described in the experimental section.

NII-Electronic Library Service

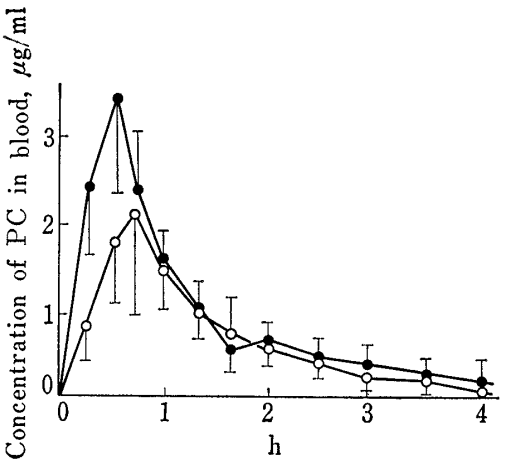


Fig. 3. Blood Levels of Ampicillin in Rabbits following Oral Administration of ABPC Na at the Dose of 15 mg/kg (—○—) and ABPC EtAA Na at the Dose of 19.6 mg/kg (●)

Each point represents the mean ± S.D. for four rabbits.

Oral Administration

The bioavailabilities of the antibiotics were studied following oral administration of the parent antibiotics or their enamine derivatives to rabbits in the form of hard gelatin capsules (Fig. 3). The results are also presented in Table V.

Upon bioautographic analysis of blood samples, only the parent antibiotics were detected in the blood following the oral administration of enamine prodrugs, so the values of bioavailability were routinely obtained from AUC of oral administration and AUC of intravenous administration of the parent drug.[16] The values of pro/parent were obtained from the ratio of the bioavailability of the prodrug to that of the parent drug.

The bioavailability in ABPC Na by oral administration was not much improved by the introduction of the enamine moiety at the amino group in the antibiotic. However, the bioavailability of AMPC·$3H_2O$ was considerably improved by the introduction of the enamine moiety. It is interesting that AMPC Na was readily absorbed by the oral route, having a bioavailability 1.5 times larger than that of the trihydrate. The reason for this was not determined in the present study, but it was considered possible that enhanced dissolution of AMPC Na played an important role in the initial stage of the gastrointestinal absorption processes. The bioavailability of CEX·H_2O was slightly improved by the use of the enamine prodrug. The statistically insignificant improvement in the bioavailability of CEX obtained by use of the enamine prodrug is presumably mainly attributable to the intrinsic high bioavailability of the parent drug.

Table V. Oral Administration of Antibiotics and Their Enamines

	Peak level μg/ml	Peak time min	Bioavailability %	Pro/parent
ABPC Na	2.1±1.1	35	19.7± 3.5	
ABPC EtAA Na	3.4±1.1	30	36.4±12.1	1.8
AMPC·$3H_2O$	2.1±0.5	50	26.8± 4.7	
AMPC Na	5.5±0.7	30	43.4± 4.4	1.6
AMPC EtAA Na	5.3±0.9	45	60.0± 2.1	2.4
CEX·H_2O	19.6±5.4	60	78.3±10.3	
CEX EtAA Na	19.0±3.6	150	98.6± 8.8	1.3

Dose: ABPC Na 15.0 mg/kg, ABPC EtAA Na 19.6 mg/kg, AMPC·$3H_2O$ 15.0 mg/kg, AMPC Na 13.9 mg/kg, AMPC EtAA Na 19.3 mg/kg, CEX·H_2O 50.0 mg/kg, CEX EtAA Na 65.2 mg/kg.
The value of pro-parent means the ratio of the bioavailability of the prodrug to that of the parent drug.
Each number represents the mean ± S.D. for four rabbits.
Sodium amoxicillin (AMPC Na) was prepared as described in the experimental section.

During oral absorption, enamine prodrugs will be exposed to the strongly acidic gastric juice and will be rapidly hydrolyzed to the parent drugs in the stomach. The present results of the improved bioavailability of enamine prodrugs may not be attributed only to the improved lipophilicity of prodrugs. The liberation and coexistence of EtAA, one of the hydrolysis products of the enamine prodrug, should also be taken into consideration. However,

NII-Electronic Library Service

concomitant oral administration of EtAA and amino acid-like β-lactam antibiotics was not studied in the present work because of the complexity arising from possible enamine formation during the absorption processes, especially in the intestinal tract.

For the study of oral administration in the fasted condition, rabbits were not suitable because of the difficulty in emptying the stomach contents. To control the stomach emptying, comprehensive feeding were required.[17] Thus, the bioavailability of ABPC EtAA Na was studied in dogs in the fasted state.

Still, a statistically significant improvement was not obtained between the prodrug and the parent drug. In order to prevent the rapid hydrolysis of the enamine prodrug in the gastric juice, enteric coated capsules were administered to dogs, but no significant increase of bioavailability was observed.

From these observations, it can be concluded that the enamine prodrugs studied are not appropriate for oral administration due to their rapid hydrolysis even at pH 6.5 (the pH of the upper part of the small intestine) before absorption, in spite of their improved lipid affinities.

Rectal Administration

Numerous investigations have demonstrated the advantages of suppositories over oral dosage forms.[18] In addition, the rectal administration of enamine prodrugs is considered to be desirable because of the avoidance of rapid hydrolysis prior to reaching the site of absorption which is unavoidable in oral administration without an appropriate preparation design. Rapid permeation through the rectal membrane immediately after the release of the drugs from suppositories to the rectal juice at neutral pH[19] is also expected for the enamine prodrugs. Thus, the rectal administration of the enamine prodrugs was studied.

Many workers have demonstrated the importance of lipid affinity of drugs for permeation through the rectal membrane.[20]

As a preliminary experiment, β-lactam antibiotics having various lipid affinities were administered to the rectum of rabbits in liquid paraffin-white petrolatum base. The results

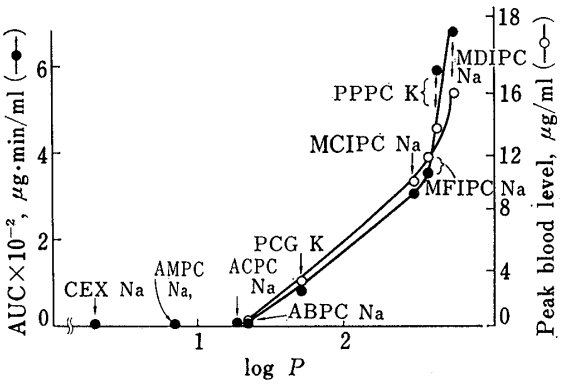


Fig. 4. Relationship between the Rectal Absorption of Antibiotics in Rabbits and Their Lipid-Water Partitioning Properties (log *P*)

Abbreviations in the figure are sodium or potassium salt of dichloxacillin (MDIPC Na), propicillin (PPPC K), fluchloxacillin (MFIPC Na), chloxacillin (MCIPC Na), penicillin G (PCG K), and cyclacillin (ACPC Na). Each point represents the mean value for five to six rabbits. Each antibiotic was suspended in liquid paraffin-white petrolatum base (50—50 w/w) at the concentration of 10 w/w%.

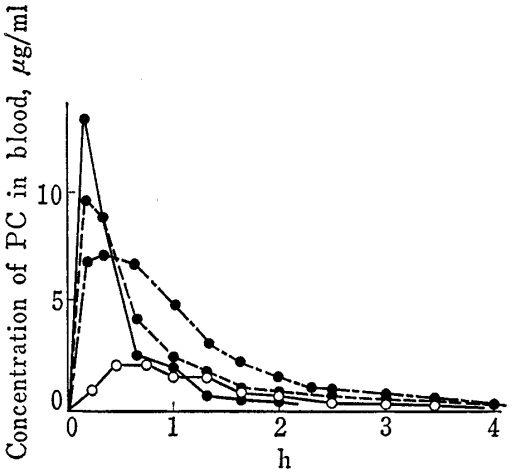


Fig. 5. Blood Levels of ABPC in Rabbits following the Rectal Administration of ABPC EtAA Na at a Dose of 19.6 mg/kg suspended in Witepsol H-15 (—●—), Liquid Paraffin-White Petrolatum (···●···), or Squalane-White Petrolatum (—·●·—)

The results of oral administration of ABPC Na to rabbits are also presented (—○—).
Each point represents the mean for four rabbits.

NII-Electronic Library Service

are plotted in terms of values of AUC and peak blood level, C_{max}, against the lipid affinity, log P[21] in Fig. 4.

The logarithms of AUC and C_{max} were found to be linearly correlated with log P by the following equation:

$$\log \text{AUC} = 0.972 \log P + 0.147 \ (n=5, r=0.9936)$$
$$\log C_{max} = 0.772 \log P - 0.780 \ (n=5, r=0.9998)$$

where n and r indicate the number of antibiotics studied and the correlation coefficient, respectively.

ABPC Na, ACPC Na, AMPC Na and CEX Na could not be detected in the blood following rectal administration at the dose of 15 mg/kg, suggesting the importance of lipid affinity or oil-water partitioning for the rectal absorption of antibiotics. Many suppositories bases do not seem to be adequate for the preparation of suppositories of β-lactam antibiotics because of considerable degradation during storage.[22] Thus, the mixture of liquid paraffin and white petrolatum was mainly used as the base in the present study.

Suspensions of ABPC EtAA Na were prepared in three bases and administered to the rectum of rabbits at the same dose as in the oral administration experiments. The results are presented in Fig. 5.

For the sake of comparison, the results of oral administration of ABPC Na are also presented in Fig. 5. The bioavailability of ABPC was significantly improved by the rectal administration of ABPC EtAA Na over that of the oral administration of ABPC Na. The results of rectal administration of enamine prodrugs studied are presented in Table VI in terms of peak level, time required to reach peak level, apparent bioavailability compared with intravenous administration of the parent antibiotics, and relative bioavailability with respect to oral administration of the parent antibiotics, *p.r./p.o.* Two commercially available prodrugs

Table VI. Results of Rectal Administration of Antibiotics and Their Enamines

Vehicle	Peak level μg/ml	Peak time min	Bioavailability %	*p.r./p.o.*
		ABPC EtAA Na		
Witepsol H-15	13.2±3.7	10	45.9± 9.3	2.3
Migryol 812	14.7±1.5	10	58.0± 7.6	2.9
1/2 liquid paraffin 1/2 white petrolatum	10.1±2.3	10	63.8±11.3	3.2
1/2 squalane 1/2 white petrolatum	8.6±3.7	20	85.0± 9.5	4.3
		AMPC EtAA Na		
Witepsol H-15	7.2±2.2	10	29.7± 8.1	1.1
1/2 liquid paraffin 1/2 white petrolatum	10.3±4.8	10	62.1±17.7	2.3
		CEX EtAA Na		
Witepsol H-15	8.6±0.3	60	34.0± 5.0	0.4
1/2 liquid paraffin 1/2 white petrolatum	14.0±2.1	50	63.2± 4.2	0.8
		TAPC·HCl		
1/2 liquid paraffin 1/2 white petrolatum	2.3±0.8	50	36.2± 6.0	1.8
		IPABPC K		
1/2 liquid paraffin 1/2 white petrolatum	0.5±0.1	30	3.5± 1.3	0.2

Dose: ABPC EtAA Na 19.6 mg/kg, TAPC·HCl 20.8 mg/kg, AMPC EtAA Na 19.3 mg/kg, IPABPC K 17.3 mg/kg, CEX EtAA Na 65.2 mg/kg.
Rp: PC: 0.1 g+vehicle: 0.9 g, CEP: 0.2 g+vehicle: 0.8 g.
The value of p.r/*p.o.* means the ratio of the bioavailability of the prodrug after rectal administration to that of the parent drug after oral administration.
Each number represents the mean±S.D. for four rabbits.

NII-Electronic Library Service

of ABPC, *i.e.*, TAPC·HCl and IPABPC K were also rectally administered to rabbits. The results are included in Table VI.

In general, enamine prodrugs of antibiotics studied were found to be adequate for rectal administration. Their rectal absorption was considerably influenced by the base used, and in rabbits the hydrocarbon bases were found to be more suitable than the triglyceride bases. TAPC·HCl was found to be effectively absorbed, but the preparation had an expulsive effect on many rabbits, suggesting considerable irritation to the rectal membrane because of the strong acidity of the hydrochloride. The reasons for poor absorptivity of IPABPC K were not determined.

To study the hydrolysis of enamine prodrugs during the absorption process and in the blood stream after rectal administration, blood concentrations of ABPC EtAA and ABPC were measured by means of bioautographic analysis. However, no enamine fraction was detected in blood samples. Thus, the presence of enamine prodrug in the blood stream need not be considered at the low dose of 15 mg/kg. At such a low dose, hydrolysis of the enamine moiety seems to be performed during passage through the rectal membrane. As expected from the results of the stability study, an enamine prodrug is hydrolyzed to some extent even in the rectum. In spite of this disadvantage, the absolute bioavailability of ABPC from the enamine prodrug was excellent in rabbits. This finding suggests that the enamine prodrug may also promote the absorption of ABPC derived from the prodrug in the rectum.

To confirm this, mixtures of ABPC EtAA Na and ABPC Na were suspended in the hydrocarbon base and administered to the rectum of rabbits to a dose equivalent to 15 mg/kg of ABPC Na. The results are presented in Fig. 6(a) and (b).

In Fig. 6(a), it can be seen that a mixture of 7.5 mg/kg of ABPC Na and 9.8 mg/kg of ABPC EtAA Na (equal amounts on a molar basis) resulted in the same blood level profiles as 19.6 mg/kg of the prodrug alone (same dose as 15 mg/kg of ABPC Na on a molar basis), while a dose of 9.8 mg/kg of ABPC EtAA Na alone (same dose as administered in the above

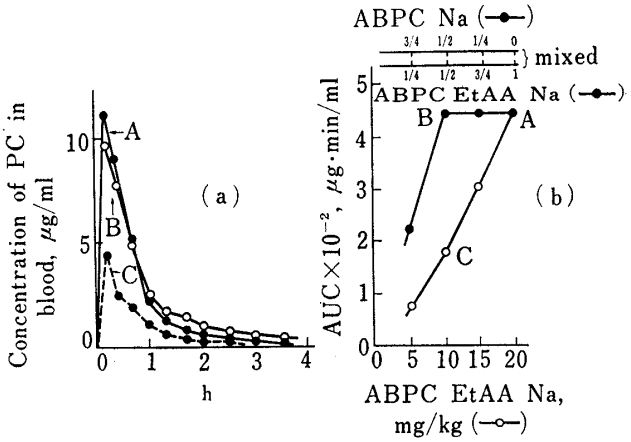


Fig. 6(a). Blood Levels of ABPC in Rabbits following Rectal Administration of ABPC EtAA Na(A) (—●—), a Mixture of 1/2 ABPC EtAA Na and 1/2 ABPC Na(B) (—○—), or 1/2 ABPC EtAA Na(C) (···●···) at a Dose Equivalent to 15 mg/kg of ABPC Na.

6(b). Relationship bet ween the Values of AUC and the Mixing Ratioof the Two Antibiotics or the Fraction of ABPCEtAA Na mixed in the Preparation

—○—: the dose-AUC profiles of ABPC EtAA Na alone; —●—: the mixing ratio-AUC profiles for concomitant administration of the two antibiotics. The points represented by A, B, and C in 6(b) correspond to the values of AUC obtained from the corresponding blood levels in 6(a).

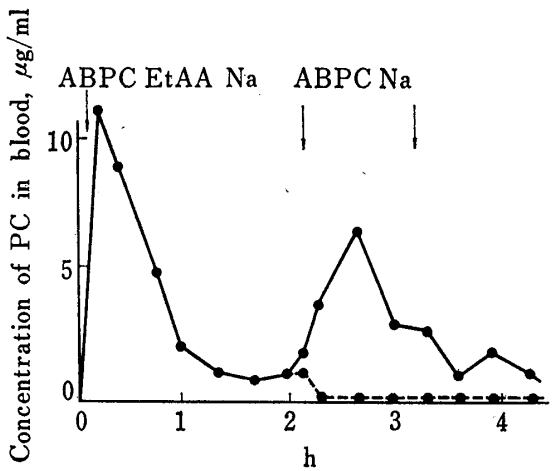


Fig. 7. Blood Levels of ABPC in Rabbits following the Initial Rectal Administration of ABPC EtAA Na (19.6 mg/kg) and Two Consecutive Administrations of Suppositories with (—●—) or without (···●···) ABPC Na at 2 and 3 h after the Initial Dosing

Liquid paraffin-white petrolatum base was used. Each point represents the mean for three rabbits.

NII-Electronic Library Service

experiment in terms of ABPC EtAA Na) showed a lower blood concentration profile than that expected from the dose.

These results were considered to suggest possible simultaneous absorption of ABPC Na promoted by the coadministration with ABPC EtAA Na. To study the promoting effect of ABPC EtAA Na on the rectal absorption of ABPC Na, various mixtures of the two drugs were administered to rabbits. The results are presented in terms of the values of AUC in Fig. 6. The values of AUC following the rectal administration of ABPC EtAA Na at doses of 4.9—19.6 mg/kg were found to be linearly correlated with the dose, suggesting that the permeation of the prodrug was passive. The differences in the values of AUC between ABPC EtAA alone and the mixture of two drugs are attributable to additional ABPC Na absorption due to the promoting effect of ABPC EtAA Na.

From these results, it may be considered that possible hydrolysis of the enamine prodrug in the rectum does not influence the apparent bioavailability of the prodrug.

To study the duration of the promoting effect of ABPC EtAA Na on the rectal absorption of ABPC Na, a suspension of the prodrug in the hydrocarbon base was administered rectally to a rabbit as an initial dose and the hydrocarbon base alone or a suspension of ABPC Na in the base was administered 2 and 3 h after the initial dosing (Fig. 7). The administration of the base alone was found to have no effect on the declining blood level of ABPC, suggesting that further absorption of ABPC EtAA Na remaining in the rectum did not occur upon insertion of the base alone. However, upon administration of ABPC Na suspension 2 h after the initial dosing, a significant increase in the blood level was observed, but further administration at 3 h failed to increase the blood level. These results suggest that the effect of ABPC EtAA Na on the rectal membrane disappears within 3 h after the initial administration.

The mechanism by which ABPC EtAA Na promotes the rectal absorption of ABPC Na itself was not clarified in the present study.

In conclusion, the enamine prodrugs of amino acid-like β-lactam antibiotics are suitable for rectal administration. In view of their improved bioavailability as compared with oral administration of the parent drugs, these enamine derivatives may be practically useful prodrugs.

Acknowledgement The authors thank Prof. I. Kitagawa of Osaka University for advice and discussions which were helpful in the development of this work.

References and Notes

1) Part of this work was presented at the 97th and 98th Meetings of the Pharmaceutical Society of Japan, Tokyo, April 1977 and Okayama, April 1978, respectively.
2) Location: *a) 133-1 Yamada-Kami, Suita, Osaka, 565, Japan*; *b) 1-2-3 Kasumi, Hiroshima, 734, Japan.*
3) W.M.M. Kirby and C. Kind, *Ann. N. Acad. Sci.*, **145**, 291 (1967); Calvin M. Kunin, *ibid.*, **145**, 282 (1967); W. Daehne, E. Frederiksen, E. Gundersen, F. Lund, P. Morch, H.J. Petersen, K. Roholt, L. Tybring, and W.O. Godtredsen, *J. Med. Chem.*, **13**, 607 (1970).
4) E.D. Purich, J.L. Colaizzi, and R.I. Poust, *J. Pharm. Sci.*, **62**, 545 (1973).
5) T. Higuchi and U. Stella, "Pro-drugs as Novel Drug Delivery Systems," ACS Symposium Series 14, American Chemical Society, Washington, D.C., 1978, p. 118.
6) G.A. Hardcastle, D.A. Johnson, and C.A. Panetta, *J. Org. Chem.*, **31**, 397 (1966).
7) W.J. Jusko and G.P. Lewis, *J. Pharm. Sci.*, **62**, 69 (1973).
8) M.A. Schwertz and W.L. Hay, *ibid.*, **61**, 906 (1972).
9) E. Dane and T. Dockner, *Chem. Ber.*, **98**, 789 (1965).
10) A.A.W. Long, J.H. Nayler, H. Smith, T. Taylor, and N. Ward, *J. Chem. Soc.*, **1971**, 1920.
11) H.C. Caldwell, H.J. Adams, R.G. Jones, W.A. Mann, L.W. Dittert, C.W. Chong, and J.V. Swintosky, *J. Pharm. Sci.*, **60**, 1810 (1971).
12) Hartha Windholz, "Merck Index," 9th edition, No. 3686, Merck & Co., Inc., 1977.
13) A. Kirshbaum and B. Arret, *J. Pharm. Sci.*, **56**, 511 (1967).
14) E.J. Vandamme and J.P. Vaets, *J. Chromatogr.*, **71**, 141 (1972).
15) J.M. Padfield and I.W. Kellaway, *J. Pharm. Sci.*, **62**, 1621 (1973).

NII-Electronic Library Service

16) J.G. Wagner, "Fundamentals of Clinical Pharmacokinetics," Drug Intelligence Publications, Hamilton, Illinois, 1975.
17) T. Maeda, H. Takenaka, Y. Yamahira, and T. Noguchi, *J. Pharm. Sci.*, **66**, 69 (1977).
18) H.S. Bean, A.H. Beckett, and J.E. Careless, "Advances in Pharmaceutical Sciences IV," Academic Press, Inc., London, 1967.
19) S. Muranishi, *Yakkyoku*, **18**, 213 (1967).
20) K. Kakemi and S. Muranishi, *Chem. Pharm. Bull.*, **13**, 861, 969, 976 (1965).
21) T. Yamana, T. Tsuji, E. Miyamoto, and O. Kubo, *J. Pharm. Sci.*, **66**, 747 (1977).
22) "Manufacturer's Manual of Witepsol® for Suppositories," Dynamit Nobel Chemicals, Troisdorf-Oberlar West Germany, P. 23.

NII-Electronic Library Service