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Compatibility of Cephacetrile in the Intravenous Admixtures

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Abstract. The compatibility of cephacetrile with 72 kinds of the additives was studied in 50 ml of 5% glucose solution. Reaction wtith sodium hydroxide resulted in desacetylcephacetrile, highly polar product with β-lactam cleavage and reddish purple pigment. The formation of the pigment was remarkable in neutral pH. The additives which were considered to be incompatible in 2% cephacetrile concentration because of the extensive degradation were as follows; Diamox®, Futraful®, Kanamycin sulfate®, Meylon®, Stronger Neo Minophagen C®, Neophyllin®, Viccillin®, Vistamycin®, Urokinase® and Proteamin 12X®. The degradation was also extensive even in the 10-fold diluted admixtures, suggesting that the degradation reaction is dominantly a specific acid-base catalyzed hydrolysis. White turbidity was found in the admixtures of FOY®, Novamin® and Wintermin®. From the elemental analysis and IR spectrum, the reaction product was proved to be the slightly soluble salt with equimolar composition of cephacetrile and mesyl gabexate.

Key Words: Antibiotics; cephacetrile, compatibility, intravenous admixture

Introduction

Cephacetrile is a semisynthetic cephem antibiotics having the broad antibacterial spectrum as other cephems. Its action is bactericidal. The physico-chemical properties [1] and the degradation kinetics in aqueous solution[2] have already been reported. β-Lactam antibiotics are often used with additives in infusion. Vitamins are drugs most freqently admixed. In this study, the compatibility of sodium cephacetrile with 72 kinds of additives was examined with respect to the stability in 5% glucose solution containing vitamin B complex.

For the bioassay of cephacetrile which has been performed in many reports[3-5], separation of intact cephacetrile from the desacetylproduct is necessary. The desacetyl product also has antibacterial activity although less active than parent antibiotic as seen in cephalothin and cephaloglycin. Fugono and Maeda[6] reported the simultaneous determination by bioautography using *Bacillus subtilis* ATCC 6633 as the test organism. The determination by high performance liquid chromatography was also reported[7]. However, thin layer chromatography (TLC) was found to be convenient for the rapid and precise

determination of intact cephacetrile. Therefore, the residual cephacetrile in the admixture at each time interval was followed by TLC method.

Experimentals

Materials and apparatus

Sodium cephacetrile injection (Celtol®, Lot No. OK 047) and the standard sample of sodium cephacetrile (920 μg/mg potency, Lot No. CT-26, 41/77) were supplied from Takeda Chemical Industries Co. Ltd. and used without further purification. The additives used in this study were those used in our hospital. Other chemicals were all of reagent grade. pH was measured by a Hitachi-Horiba Model F-7 pH meter. IR spectra were obtained by a Hitachi Model EPI-G 3 infrared spectrophotometer. TLC was carried out either on the glass plate (20×10 cm) which was coated with Wakogel B-5 FM in the depth of 0.25 mm and activated at 105°C for 1 h or on the precoated plate (silica Gel 60 F_{254}, Merck Japan, Tokyo).

Degradation of cephacetrile in alkaline solution

Two ml of the sodium cephacetrile aqueous solution (5.5×10^{-2}M) was reacted with equal, twice and thrice amount of 1 N sodium hydroxide solution for 15 min at room temperature, and the reaction products were detected by TLC using the solvent B described below.

Compatibility test

Sodium cephacetrile (1 g) was dissolved in 50 ml of 5% glucose solution containing vitamin B complex, namely thiamine hydrochloride injection (10 mg/ml, Metabolin G®, Takeda), sodium pyridoxal phosphate injection (10 mg/ml, Pydoxal®, Chugai) and flavin adenine dinucleotide (10 mg/ml, Flavitan®, Toaeiyo). Then, the additive was added to make the test admixture. When the additive was in the form of powder or lyophylized preparation, both sodium cephacetrile and the additives were simultaneously dissolved in the glucose-vitamin B complex admixture. When the compatibility was examined in infusions, 5% glucose solution was replaced by test infusions. The test admixtures were allowed to stand for 24 h at ambient temperature (25–27°C), and the pH and the residual concentration of cephacetrile were measured at 0, 6 and 24 h. For the additives described below, the compatibility was examined at a concentration of 0.2%, the concentration usually applied in clinical practice.

Determination of cephacetrile

TLC method: Two to 4 μl of the test admixture was spotted on the hand made TLC plate and developed by either of the solvents according to the components of the test admixture (Solvent A: chloroform-tetrahydrofuran-ethanol-formic acid (10 : 10 : 10 : 0.3); solvent B : benzene-ethylacetate-ethanol-formic acid (2 : 3 : 4 : 0.25). After developing, the spots on each TLC plate were visualized under UV light. The spot corresponding to cephacetrile was removed by scrapping with microspatula and collected in the test tube. Cephacetrile was extracted with 5 ml of purified water by shaking with vortex mixer for 5 s. After centrifugation for 10 min at 3000×*g*, the absorbance at 260 nm of the supernatant was measured. All of the measurements were performed in triplicate.

Bioassay: Agar cup plate diffusion method was applied using *Bacillus subtilis* ATCC 6633 as the test organism. Growth inhibition zones were determined for three standard solutions and one sample solution. The concentration of cephacetrile was determined from the regression line for each plate. The determination was performed in triplicate.

Precipitation product of sodium cephacetrile with mesyl gabexate

Equal volume of 0.138 M sodium cephacetrile solution (pH 4.62) and 0.138 M mesyl

gabexate (pH 4.55) were admixed. The white turbid solution (pH 4.55) that occured was left over night in refrigerator. The precipitate was collected on filter paper and washed with purified water throughly, dried *in vaccuo* and subjected to elemental analysis.

Results

Analytical method: Fig. 1 shows the thin layer chromatogram of cephacetrile and its degradation products. Cephacetrile was clearly separated with Rf 0.28 and 0.56 in solvents A and B, respectively. The regression lines of the calibration graphs up to 30 mg/ml obtained using the two solvent system were as follows: solvent A: [mg/ml]=114.95 $\times Abs_{260nm}-1.014$ ($r=0.999$, $n=24$, sample size 2 μl); [mg/ml]=57.776 $\times Abs_{260nm}+0.249$ ($r=0.998$, $n=24$, sample size 4 μl); solvent B: [mg/ml]=113.635 $\times Abs_{260nm}-1.004$ ($r=0.990$, $n=24$, sample size 2 μl); [mg/ml]=60.258 $\times Abs_{260nm}+0.357$ ($r=0.998$, $n=18$, sample size 4 μl). The coefficient of variation in the determination of 2% solution of cephacetrile were 2.7% by both solvents ($n=20$). In the experiments with 0.2% cephacetrile, the degradation was also followed by bioassay. As shown in Fig. 2, there was significant correlation between the degradation ratios determined by TLC and bioassay ($r=0.973$, $p<0.01$).

Degradation products of cephacetrile in alkaline solution: Fig. 3 shows thin layer chromatogram of the reaction solution of cephacetrile admixed with varying amounts of sodium hydroxide. The degradation of cephacetrile was incomplete in the equimolar admixture. With the twice amount of sodium hydroxide, the spot of cephacetrile completely disappeared, and desacetylcephacetrile and original spots concomitantly increased. With the thrice amount of sodium hydroxide, the spot of desacetylcephacetrile also disappeared with further increase of original spot, and new spot of red pigment[1,8] appeared. The IR spectrum of methanol extract of the original spot revealed the characteristic absorption of $\nu_{C\equiv N}$ at 2200 cm^{-1}. The main component in this spot was supposed to be the unstable degradation product resulted from β-lactam cleavage[9]. The chromatogram of each reaction solution was identical inde-

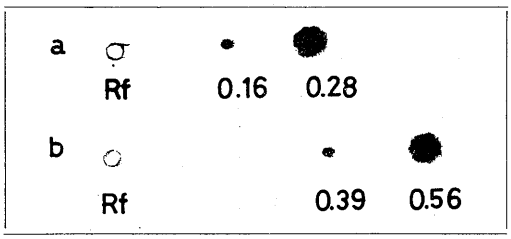

Fig. 1 Thin layer chromatogram of cephacetrile and desacetylcephacetrile.
a: Choloroform-tetrahydrofuran-ethanol-formic acid (10 : 10 : 10 : 0.3); b: benzene-ethylacetate-ethanol-formic acid (2 : 3 : 4 : 0.25); cephacetrile: Rf 0.28, 0.56; desacetylcephacetrile: Rf 0.16, 0.39.

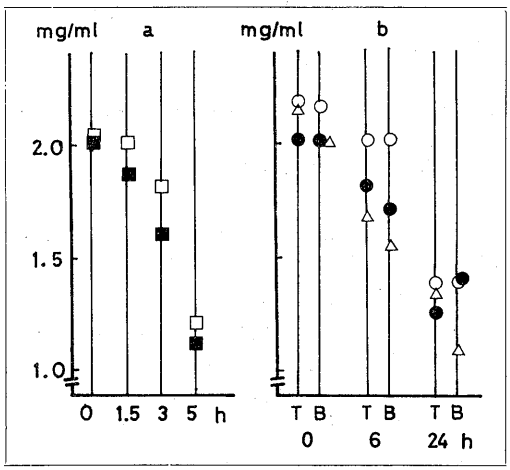

Fig. 2 Comparison of assay results by TLC with those by bioassay.
a: 0.2% of sodium cephacetrile in phosphate buffer at 40–50°C (0.1 *M*, pH 6.96–6.90, $\mu=0.6$), (□): TLC; (■): bioassay; b: 0.2% of sodium cephacetrile in the admixtures at 25–27°C; (●): Meylon®; (○): 5-FU®; (△): Diamox®; T: TLC; B: bioassay.

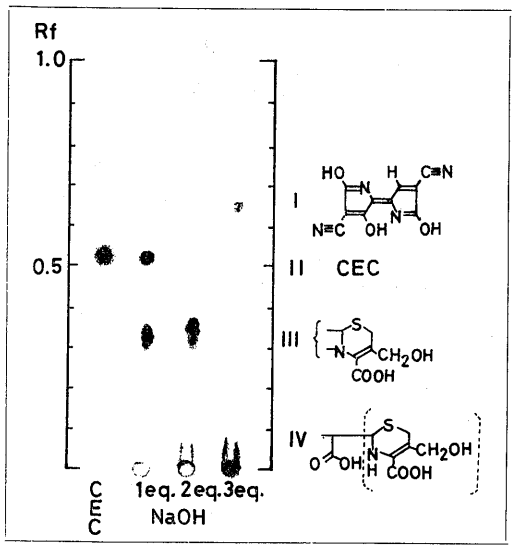

Fig. 3 Degradation products of cephacetrile in alkaline solution.
TLC: Silicagel 60F 254 (Merk); solvent: B.

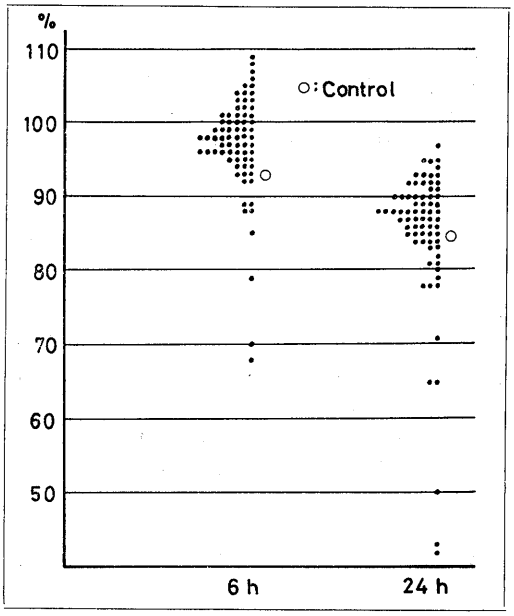

Fig. 4 Residual cephacetrile in the admixtures. Control: 2% aqueous solution

pendently on incubation times of 15 and 60 min, suggesting that the hydrolysis was quite rapidly completed.

Stability of cephacetrile in the admixture: The residual cephacetrile in the 72 kinds of admixtures are illustrated in Fig. 4. The concentration of 2% of cephacetrile, which is 10-fold higher than that in practical use, was used in this study for the analytical convenience. We regard the additive with which more than 10% of cephacetrile were degraded in 6 h as incompatible. From the pH-rate profile and activation energy obtained in previous work[2], the residual ratio at pH 4-6 at the temperature of this study (25-27°C) was predicted to be 93.2%. Therefore, the residual ratio of 80% was taken as the criteria for the incompatibility at 24 h. As obvious from Fig. 4, 11 additives were considered to be incompatible from either of the above criteria (Group A and B in Table 1). The compatibility of these additives was reexamined in the admixture with 0.2% cephacetrile, the concentration applied in practice (Table 1). Similar results with respects to change in pH and degradation of cephacetrile were obtained to those with 2% cephacetrile admixtures, although the tendency of slight decrease in the degradation ratios were apparent in 0.2% cephacetrile admixtures.

Precipitation product of cephacetrile with mesyl gabexate: White turbidity was observed in FOY®, Wintermin® and Novamin® admixtures (Group C in Table 1). The IR spectrum of the product of cephacetrile with mesyl gabexate differed apparently from those of the parent compounds, having the characteristic absorption of carboxylate ion ($-COO^-$) at 1607 cm^{-1} which appears at 1740-1720 cm^{-1} as unionized carboxylate (-COOH) in cephacetrile. The result of elemental analysis was as follow: calcd. for $C_{29}H_{35}N_{10}S$: C 52.8%, H 5.35%, N 12.75%; found: C 52.5%, H 5.49%, N 12.41%, confirming the equimolar composition of cephacetrile and gabexate.

Table 1 The pHs and degradation ratios in the 2% and 0.2% admixtures.

Additives*	pH and residual amount (%) at the concentration					
	2%			0.2%		
	0 h	6 h	24 h	0 h	6 h	24 h
Group A						
Diamox	9.05 100	8.30 67.8	7.84 42.4	9.04 100	8.31 75.3	7.75 62.1
Futraful	9.63 100	8.30 69.5	7.79 42.9	9.42 100	8.32 79.5	7.98 57.6
Meylon	7.95 100	7.99 69.5	7.72 42.9	8.05 100	8.03 75.6	7.97 70.0
Urokinase	5.70 100	4.92 92.2	4.52 78.5	5.30 100	4.46 96.9	4.30 86.5
Group B						
5-FU	8.25 100	8.11 91.5	7.70 50.4	8.17 100	— 93.3	7.58 63.3
Kanamycin sulfate	6.55 100	6.39 88.7	6.04 78.3	6.10 100	5.90 97.3	5.52 67.9
Neophyllin	8.60 100	7.65 79.4	6.78 64.7	8.18 100	7.31 89.7	6.55 78.9
Proteamin 12X	6.21 100	6.17 93.2	6.00 79.0	6.22 100	6.18 96.1	6.13 88.0
Stronger Neo Minophagen C	5.80 100	5.05 88.5	4.69 77.8	5.67 100	4.67 88.5	4.30 77.8
Viccillin	8.35 100	7.88 84.7	7.25 70.7	7.98 100	7.69 90.8	7.22 72.4
Vistamycin	6.50 100	6.17 88.4	5.81 84.6	5.22 100	5.15 91.4	5.06 80.1
Group C						
FOY	5.02 100	4.55 96.3	4.32 90.0			
		turbidity				
Novamin	5.14 100	4.70 98.4	4.41 84.0			
		turbidity				
Wintermin	5.20 100	4.80 98.9	4.51 88.2			
		turbidity				

*: brand name

Discussion

Chemical assays for the determination of cephacetrile include iodometry[1,10], hydroxamic acid method[11] and HPLC[7] as used in other β-lactam antibiotics.

Iodometry and hydroxamic acid method cannot distinguish desacetylcephacetrile from intact one. Furthermore, the lactone moiety of the degradation product also reacts with hydroxylamine to form hydroxamic acid similar to intact β-lactam. Therefore, chromatographic methods are preferred. Although HPLC is the most selective and exact method, TLC can well be used as a substitute if the separation is sufficient. Thus, the TLC method has been used even for kinetic

study[12] in which precise assay of reactant is essential. TLC method is useful because of its low cost compared to HPLC and fairly good selectivity. Combining with chromatoscanner, it would be comparable to HPLC.

The hydrolysis catalyzed by hydroxide ion is supposed to occur primarily at acetylhydroxymethyl group on C_3 and β-lactam ring and less probably at amide bond on C_7. Therefore, the complete clevage of β-lactam does not occur with equimolar amount of sodium hydroxide (Fig. 3). Fig. 3 also indicates that desacetylation is predominant over the cleavage of β-lactam, and the β-lactam of most cephacetrile molecules is cleaved after desacetylation. Red pigment have been shown to occur via β-lactam cleavage[9]. The formation is maximum at neutral pH.

The 11 additives were incompatible in the admixtures with 10-fold higher concentration than usual use. To see the compatibility of these additives in ordinary condition, the stability of cephacetrile was examined at 0.2% concentration (Table 1). The admixtures in group A are those with high pH except for Urokinase®. Hydrolysis is considered to be a dominant degradation reaction. The pHs of these diluted admixtures were still high, and these admixtures showed the extensive degradation of cephacetrile as in 2% admixtures. Urokinase® contains phosphate which is considered to stimulate the degradation[13].

The additives in group B contain the components having primary amine moiety. The reaction with β-lactam to make amide products are supposed to occur in addition to hydrolysis. Ethylenediamine[14] as a molecular component of aminophylline (Neophyllin®), tromethamine[14b,15], a solubilizing agent of 5-fluorouracil (5-FU®), and cysteine and glycine[16] in Stronger Neo Minophagen C® are the components concerned. Similarly, the reaction with amino acids are also anticipated[17] in Proteamin 12X® admixture. The aminoglycoside antibiotics, kanamycin and ribostamycin[18], may also react with cephacetrile and the reaction with aminobenzyl group is also possible in ampicillin admixture[19].

As reported by Yamana et al[20], aminolysis of β-lactam is second order and/or accompanied with higher order reactions regarding the concentrations of β-lactam antibiotics and amines. Therefore, the dilution of components by ten-fold may result in remarkable decrease of reaction rate. For example, the residual ratio of 50% in 5-FU® admixture with 2% cephacetrile concentration should be 90% in 0.2% admixture as calculated by Equation 1. Similarly, the residual ratio of 70% in 2% admixture should be 96% in 0.2% in the case of Viccillin® admixture.

$$A/A_0=1/(1+kA_0t) \quad \cdots\cdots (1)$$

Where A is the amount at time t; A_0 is the initial amount; k is the pseudo second order rate constant.

But the decrease of degradation was not so remarkable in these admixtures, suggesting that the degradation of cephacetrile predominantly depends on the hydrolysis and the contribution of aminolysis reaction is smaller.

Another interaction of β-lactam antibiotics with amines are the formation of a complex and/or salt[21]. In this study, white turbidity was observed in the admixtures of FOY®, Novamin® and Wintermin® (Group C in Table 1). The product with mesyl gabexate, a component of FOY®, was confirmed to be an organic salt resulted from the neutralization between the carboxyic acid moiety of cephacetrile and guanyl group of gabexate. In the case of Novamin® and Wintermin®, similar interaction, the formation of salt with low water solubility, seems likely to occur as already reported[21].

Thus, causion should be paid not only to aminolysis but also to the formation of a complex and/or salt with low water soluble property when admixed with amine. Most of these interaction are, however, easily avoided by dilution of the components.

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References

1) Nakajima, N., Yoshida, K., Asahi, Y., Okada, Y., Oda, Y and Yoshinari, T.: Physico-chemical properties and stability of cephacetrile sodium (in Japanese). *J. Takeda Res. Lab.*, 34 : 369-382, 1975.

2) Koshiro, A. and Fujita, T., Kinetic study of the degradation of cephacetrile in aqueous solution by TLC. *Yakuzaigaku*, 43 : 36-42, 1983.

3) Knusel, F., Knopka, E.A., Gelzer, J. and Raselet, A., Antimicrobial studies in vitro with CIBA 36, 278-Ba, a new cephalosporin derivatives. *Antimicrob. Agents Chemother.*, 1 : 140-149, 1971.

4) Nissenson, A.R., Nathan, M.D., Levin, W. and Parker, R.H., Effect of renal failure and hemodialysis on cephacetrile pharmacokinetics. *Clin. Pharmacol. Therap.*, 13 : 887-894, 1972.

5) Hodges, G.R., Scholand, J.F. and Perkins, R.L., Cephacetrile clinical evaluation in 27 patients. *Antimicrob. Agents Chemother.*, 3 : 228-234, 1973.

6) Fugono, T. and Maeda, K., Differential assay method for cephacetrile and its metabolites (in Japanese). *Chemotherapy* (Tokyo), 24 : 78-84, 1976.

7) Mangia, A., Silingardi, S., Bortesi, F., Grisanti ,G. and Bitetto, M., High performance liquid chromatographic determination of cephacetrile. *J. Pharm. Sci.*, 68 : 652-654, 1979.

8) a) Meyer-Brunot, H.G., Kraetz, J., Schenk, C., Thomann, P., Zak, O. and Zimmerman, W., Studies of formation of violet-reddish pigment from the cephalospolin derivatives Celospor (C 36, 278-Ba). *Chemotherapy* (Basel), 20 : 6-17, 1974.
b) Yamanaka, Y., Kawano, S., Tateishi, H. and Aratani, H., Pharmacologiacal studies on cephacetrile: Distribution, fate, and excretion (in Japanese). *Chemotherapy* (Tokyo), 24 : 62-77, 1976.

9) a) Shiho, O. and Tsuchiya, K., IgE antibodies for penicillins and cephalosporins in rats. Ⅲ antigenic specificity of rat anti-cephalosporin-OvA IgE sera *J. Antibiot.*, 34 : 84-89, 1981.
b) Aoki, I., Konishi, E. and Kuwayama, M., Physico-chemical properties and stabilities of cefsulodin sodium (in Japanese). *Iyakuhin Kenkyu*, 9 : 1022-1030, 1978.

10) Finholt, P., Jurgensen, G. and Kristiansen, H. Catalytic effect of buffers on the degradation of penicillin G in aqueous solution. *J. Pharm. Sci.*, 54 : 387-393, 1965.

11) Munson, J.W., Papadimitriou, D. and Deluca, P., Colorimetric determination of penicillins and related compounds in intravenous solutions by Nickel (Ⅱ)-catalyzed hydroxamic acid formation. *J. Pharm. Sci.*, 68 : 1333-1335, 1979.

12) a) Maulding, M.V., Polesuk, J. and Rosenbaum ,D., Practical kinetics I: Quantitation of barbiturates stability by TLC. *J. Pharm. Sci.*, 64 : 272-274, 1975; b) Maulding, M.V. and Michelis, A.F., Practical kinetics Ⅱ : Quantication of procaine stability by TLC, *J. Pharm. Sci.*, 64 : 275-278, 1975; c) Maulding, M.V., Nanzareno, J.P., Peason, J.E. and Michaelis, A F., Practical kinetics Ⅲ: Benzodiazepine hydrolysis. *J. Pharm. Sci.*, 64 : 278-284, 1975.

13) Bundgaard, H. and Hansen, J., Nucleophilic phosphate-catalyzed degradation of penicillins: Demonstaration of penicilloyl phosphate intermediate and transformation of ampicillin to piperadinedione. *Int. J. Pharm.*, 9 : 295-303, 1981.

14) a) Schwartz, M.A., Aminolysis of benzylpenicillin by aliphatic diamines *J. Pharm Sci*, 57 : 1209-1211, 1968; b) Fukuchi, H , Yoshida, M., Kumagai, M. and Kitaura, T., Stability of sulfobenzylpenicillin in large volume parenteral solutions. *Drug Intell. Clin. Pharm.*, 12 : 418-423, 1978.

15) a) Schwartz, M.A., Catalysis by poly-L-lysine of aminolysis of penicillin by tris (hydroxymethyl) aminometane. *J. Med. Chem.*, 12 : 36-38, 1969; b) Schwartz, M.A. and Delduce, A.J., Kinetic analysis of penicilloic acid and penicilloamides in combination: Application of products of reaction of penicillin with tromethamine (Tris) and poly-L-lysine. *J. Pharm. Sci.*, 58 : 1137-1139, 1969.

16) a) Yamana, T., Akira, T., Miyamoto, E. and Kiya, E., The reaction of benzylpenicillin with amine containing hydroxy group. *J. Pharm. Pharmacol.*, 27 : 771-774, 1975 ; b) Yamana, T., Mizukami, Y., Ichimura, F. and Yokogawa,

K., Stability of carbenicillin in aqueous solution of aminothiol compounds (in Japanese). *Yakuzaigaku*, 38 : 238–242, 1978.

17) Yamana, T., Mizukami, Y., Ichimura, F. and Yokogawa, K., Stability of penicillins in amino acid solutions (in Japanese). *Yakuzaigaku*, 38 : 234–237, 1978.

18) a) Yamana, T., Mizukami, Y., Ichimura, F. and Yokogawa, K., *Yakuzaigaku*, Reactions between benzylpenicillin and aminoglycoside antibiotics (in Japanese). 37 : 193–197, 1977; b) *idem, ibid.*, Reaction between β-lactam antibiotics and aminoglycoside antibiotics (in Japanese). 38 : 74–81, 1978.

19) Bundgaard, H., Spectrophotometric determination of ampicillin sodium in presence of its degradation and polymerization products. *J. Pharm. Pharmacol.*, 26 : 385–392, 1974.

20) Tsuji, A., Yamana, A., Miyamoto, E. and Kiya, E., Chemical reaction involved in penicillin allergy: Kinetics and mechanism of penicillin aminolysis. *J. Pharm. Pharmacol.*, 27 : 580–587, 1975.

21) Koshiro, A., Fujita, T., Harada, S. and Tsujii, T., Interaction of β-lactam antibiotics with tertiary amines (in Japanese). *Yakuzaigaku*, 42 : 30–36, 1982.