Estimation of the Molecular Volumes of the Reaction Products between β-Lactam Antibiotics and Aminoglycoside Antibiotics, and Those of the Degradation Products of β-Lactam Antibiotics by Isotachophoresis

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The correlative equations between the molecular volume and the qualitative indication (h_R) for β-lactam antibiotics, the reaction products between β-lactam antibiotics and kanamycin, and the degradation products of β-lactam antibiotics were $h_R = 0.32 + 0.080 V_A^{2/3}/|Z|$ ($N = 15$, $r = 0.972$ for penicillins) and $h_R = 0.04 + 0.072 V_A^{2/3}/|Z|$ ($N = 12$, $r = 0.987$ for cephems). Where V_A is van der Waals volume (Å^3/molecule), h_R is the relative step height in the isotachopherogram, and Z is the electric change, respectively.

According to these equations, the molecular volumes of the reaction products between β-lactam antibiotics and the other aminoglycoside antibiotics, and those of the degradation products of β-lactam antibiotics can be estimated from the value of h_R. Also according to the step height in the isotachopherogram, the reaction products or the degradation products may be estimated directly when the electric charge is known.

It was confirmed that a molecule of aminoglycoside antibiotics reacted with a molecule of β-lactam antibiotics. Therefore, the inactivation of aminoglycoside antibiotics is much greater than for β-lactam antibiotics when the clinical doses of these antibiotic combinations are used.

Keywords isotachophoresis; molecular volume estimation; ionic mobility; aminoglycoside antibiotic; β-lactam antibiotic; reaction product estimation; qualitative indication; degradation product estimation

In the previous study,[1] we reported the correlative equations between the molecular volume (V_A) and R_E or the absolute mobility (m_0: 10^{-5}cm^{2}V^{-1}s^{-1}) for organic anionic substances, amino acids, dipeptides and β-lactam antibiotics (cephems) *i.e.*, $R_E = a + b\, V_A^{2/3}/|Z|$, or $m_0 = a' + b'|Z|/V_A^{2/3}$. Where V_A is van der Waals volume (Å^3/molecule)[1] and R_E is the ratio of the potential gradient of the sample zone to that of the leading zone,[2] Z is the electric change of ion, and a, b, a' and b' are constants, respectively. According to one of these equations, the molecular volume of ion may be estimated from the value of R_E, when the electric charge is known, and the value of R_E or m_0 may be estimated from the molecular volume. However, these relationships contain a deviation based on the variation of the structure of ions.[3]

On the other hand, the values of the absolute mobility (m_0) of dipeptides[4] are invariably smaller than those of the constituent amino acids.[5] And similarly, the values of m_0 of β-lactam antibiotics (cephems) are invariably smaller than those of cysteine and valine,[1] because β-lactam antibiotics consist of cysteine and valine.[6] This means that the zones of dipeptides and β-lactam antibiotics in the isotachopherogram invariably follow those of the constituent amino acids when the electric charge is constant.[1]

This paper describes our studies on the application of these relationships to the qualitative analysis of the reaction products between β-lactam antibiotics (penicillins[7] and cephems[8]) and aminoglycoside antibiotics, and the degradation products of β-lactam antibiotics. The reason for our studies on these antibiotic combinations is the inactivation of both antibiotics by their interaction,[9,10] in spite of the additive or synergistic expectations of their antibacterial effects in the clinical treatment of infections.[11]

In this study we use kanamycin, an aminoglycoside antibiotic whose dosage is almost the same as that of β-lactam antibiotics, since the other aminoglycoside antibiotics, *e.g.* gentamicin, tobramycin and dibekacin *etc.*, are used at only about 10% the dosage of β-lactam antibiotics.[12,13]

Calculation

The significance of the correlation coefficient (r) was tested by means of the t-test in each case after transformation of r to z.

(1) Derivation of the Correlative Equations between the Molecular Volume (V_A) and the Relative Step Height (h_R), and V_A and R_E for Some Penicillins (PCs) The values of V_A (Å^3/molecule) of PCs were calculated from the data for some atoms or groups in the previous paper.[1] The values of h_R and R_E were obtained experimentally. h_R is defined as h_S/h_L,[14] and R_E is corrected for h_R *i.e.*, $R_E = E_S/E_L = (h_S + \Delta h)/(h_L + \Delta h)$[2,15] where h_S is the observed step height of the sample zone, and h_L is that of the leading zone. The electric charge (Z) was calculated from the equation of Kiso and Falk [16]: $Z = -1/2\sum[1 + \tanh\{2.303/2(\mathrm{pH} - \mathrm{p}K)\}]$ where pH_L is the pH of the leading electrolyte.

(2) Estimation of the Molecular Volumes of the Reaction Products between PCs and Kanamycin (KM), and Those of the Degradation Products of PCs by Isotachophoresis 0.01 M PCs aqueous solutions and 0.042 M KM aqueous solution were reacted with a buffer solution of pH 6.4 at 35 °C.[17] The reaction products between PCs and KM, and the degradation products of PCs were analyzed by isotachophoresis, experimentally. The ratio of the reaction products and the degradation products were calculated from each of the quantitative results.

(3) Derivation of the Correlative Equation between the Molecular Volume (V_A) and the Relative Step Height (h_R) for the Reaction Products between PCs and KM, PCs, and the Degradation Products of PCs The values of V_A (Å^3/molecule) of the reaction products between PCs and KM, and the degradation products of PCs were calculated from the data for some atoms or groups in the previous paper.[1] The values of V_A of PCs used are listed in Table I. The values of h_R of the reaction products between PCs and KM, PCs, and the degradation products of PCs were

obtained experimentally. The electric charge was calculated as described previously.

(4) Derivation of the Correlative Equation between Molecular Volume (V_A) and the Relative Step Height (h_R) for the Reaction Products between Cephems and KM, Cephems, and the Degradation Products of Cephems The values of V_A (Å^3/molecule) of the reaction products between cephems and KM, and the degradation products of cephems were calculated from the data for some atoms and groups in the previous paper.[1] The values of V_A of cephems were taken from the previous paper.[1] The values of h_R of the reaction products between cephems and KM, cephems, and the degradation products of cephems were obtained experimentally. The electric charge was calculated as described previously.

Experimental

Sample and Reagents The potassium salt of benzylpenicillin (PCG, M: molecular weight = 372.48, $pK_a = 2.76$),[12] the sodium salts of ampicillin (ABPC, $M = 403.45$, $pK_2 = 7.24$),[12] carbenicillin (CBPC, $M = 422.36$, $pK_a = 3.06$, 3.3),[12] cloxacillin (MCIPC, $M = 475.88$, $pK_a = 2.73$),[12] and sulbenicillin (SBPC, $M = 458.42$, $pK_1 < 1$, $pK_2 = 2.5$—2.7)[12,13] were purchased from Banyu Pharmaceutical Co., Ltd., Meiji Seika Kaisha, Ltd., Pfizer Pharmaceuticals Inc., Meiji Seika Kaisha, Ltd., and Takeda Chemical Industries, Ltd., respectively. The sodium salts of cefalotin (CET, $M = 418.41$, $pK_a = 3.6 \pm 0.13$),[12] cefapirin (CEPR, $M = 445.44$, $pK_a = ca.$ 5.3),[12,13] cefmetazole (CMZ, $M = 493.51$, $pK_a = 2.34$),[12,13] and cefotaxime (CTX, $M = 477.44$, $pK_a = ca.$ 3.4)[13] were purchased from Shionogi & Co., Ltd., Bristol-Myers Co., Sankyo Co., Ltd., and Hoechst Japan Ltd., respectively. Kanamycin sulfate (KM, $M = 484.5$)[12] was purchased from Meiji Seika Kaisha, Ltd. Hydroxypropyl cellulose (HPC, 1000—4000 cps) was purchased from Tokyo Kasei Kogyo Co., Ltd. Other reagents were purchased from Nakarai Chemicals, Ltd.

Electrolytes System for Isotachophoresis and Instruments The leading ion was 0.01 M chloride. The pH of the leading electrolyte (pH_L) was adjusted to 8.6 by using Amediol (2-amino-2-methyl-1,3-propanediol)[2,18], and 0.02% HPC was added as a surfactant in order to suppress electroendosmosis and to increase the sharpness of the zone boundaries.[4,5] The terminating ion was 0.01 M β-alanine. The pH of the terminating electrolyte (pH 11.4) was adjusted by using $Ba(OH)_2$; Ba^{2+} precipitates with CO_3^{2-} and should remove the influence of CO_3^{2-}. The migration current was 100 μA. An IP-1B isotachophoretic analyzer with a separating tube of 0.5 mm i.d. and 15 cm length, and a PGD-1 potential gradient detector (Shimadzu Seisakusho Ltd., Kyoto, Japan) were used.[19] The reaction temperature of the bath was maintained by a KP-30 thermostat (Chino, Tokyo, Japan).

Results and Discussion

(1) Derivation of the Correlative Equations between the Molecular Volume (V_A) and the Relative Step Height (h_R), and V_A and R_E for Some Penicillins (PCs) The values of R_E, h_R, V_A and the electric charge (Z) for some PCs are listed in Table I.

The following correlative equations between V_A and h_R, and V_A and R_E for some PCs were derived ($p < 0.01$).

$$h_R = 0.33 + 0.079 V_A^{2/3}/|Z| \quad (r = 0.997, N = 5) \tag{1}$$

$$R_E = -0.12 + 0.090 V_A^{2/3}/|Z| \quad (r = 0.998, N = 4) \tag{2}$$

According to these equations, the molecular volumes of the other PCs may be estimated from the value of h_R or R_E, when the electric charge is known.

The optimum pH of electrolytes for the estimation of the molecular volume or for the separation of PCs may be determined from the $h_R - pH_L$ curves[14,15] or $R_E - pH_L$ curves[4,5] obtained on the basis of these equations.

(2) Estimation of the Molecular Volumes of the Reaction Products between PCs and KM, and Those of the Degradation Products of PCs by Isotachophoresis The final reaction products of PCs and KM, and the degradation products of PCs were reported by Yamana *et al.* as follows. The reaction product is PM: *N*-(benzylpenicilloyl) kanamycin and the degradation products are the hydrolysis products, penicilloic acid (PA).[17]

As Fig. 1 shows, the molecular volumes of PA are almost the same as those of PCs, and the electric charge ($|Z|$) of PA is larger than that of PCs, since PA have one more carboxyl group by the hydrolysis of β-lactam rings of PCs. Therefore, as Eq. 1 shows, the h_R of PA in isotachophoresis is smaller than that of PCs.

On the other hand, as Fig. 1 shows, the molecular volumes of PM are larger than those of PCs, and Z of PM is the same as PCs, since the amino group of KM reacts with the β-lactam rings of PCs by the peptide reaction.[20] Therefore, as Eq. 1 shows, the h_R of PM in isotachophoresis is larger than that of PCs.

Table II shows the ratio of PM and PA, which were calculated from each of the quantitative results by isotachophoresis, and those by Yamana *et al.*, which were determined by iodometry and penamaldate assay, *etc.*[17]

These results show good agreement between the isotachophoretic results and those of Yamana *et al.*, and that the molecular volumes of the reaction products between PCs and KM, and those of the degradation products of PCs can be estimated by the relative step height in the isotachophoresis.

(3) Derivation of the Correlative Equation between the Molecular Volume (V_A) and the Relative Step Height (h_R) for the Reaction Products between PCs and KM, PCs, and the Degradation Products of PCs The values of V_A, h_R and the electric charge (Z) for the reaction products between PCs and KM, and the degradation products of PCs are listed in Table III.

The following correlative equation between V_A and h_R for the reaction products between PCs and KM ($N = 5$), PCs ($N = 5$), and the degradation products of PCs ($N = 5$) was derived ($p < 0.01$).

$$h_R = 0.32 + 0.080 V_A^{2/3}/|Z| \quad (r = 0.972, N = 15) \tag{3}$$

According to this equation, the molecular volumes of the reaction products between PCs and the other aminoglycoside antibiotics may be estimated from the value of h_R, when the electric charge is known.

Table I. Some Values for PCs

PCs	$R_E \pm$S.D.[a] ($N = 5$)	$h_R \pm$S.D.[a] ($N = 5$)	V_A[b]	$\|Z\|$[c]
ABPC	4.04 ± 0.09	3.91 ± 0.06	292.56	0.96
BPC	3.67 ± 0.04	3.82 ± 0.08	280.79	1.00
CBPC	1.95 ± 0.14	2.16 ± 0.04	300.63	2.00
MCIPC	—[d]	4.07 ± 0.04	329.42	1.00
SBPC	1.92 ± 0.02	2.10 ± 0.05	319.59	2.00

a) The experimental values. R_E is the ratio of the potential gradient of the sample zone to that of the leading zone: $R_E = E_S/E_L = (h_S + \Delta h)/(h_L + \Delta h)$.[2,15] h_R is the relative step height: $h_R = h_S/h_L$,[14] where h_S is the step height of the sample zone, and h_L is that of leading zone. *b*) The molecular volumes (V_A: Å^3/molecule) were calculated from the data for some atoms or groups.[1] *c*) The electric charge (Z) was calculated from the equation: $Z = -1/2\,\Sigma[1 + \tan h\{2.303/2(pH - pK)\}]$.[16] *d*) No date, since MCIPC reacted with the internal standard (acetic acid).

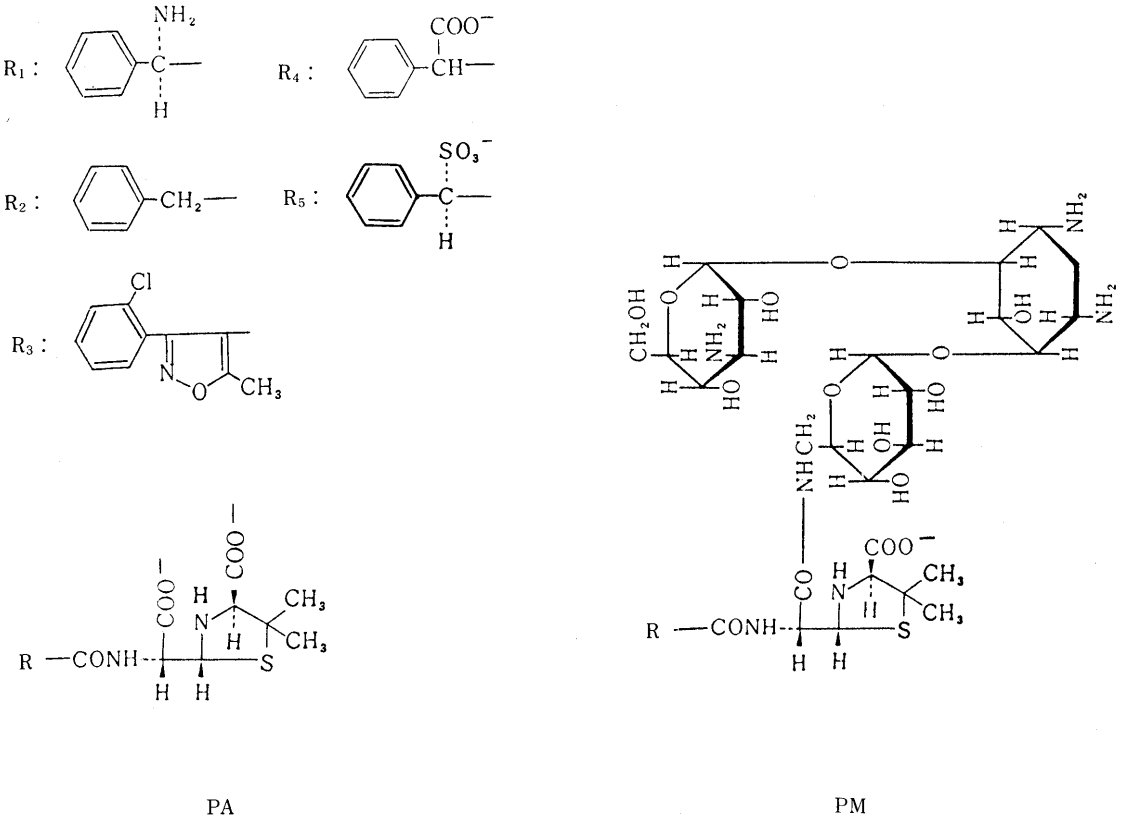

Fig. 1. The Degradation Products of PCs and the Reaction Products between PCs andKM

PA: the degradation (hydrolysis) product of PCs, the maximum electric charge ($|Z|$) of PA for monovalent PCs is 2, and Z of PA for divalent PCs is 3. PM: the reaction product between PCs and KM, Z of PM for monovalent PCs is 1, and Z of PM for divalent PCs is 2. Monovalent PCs are R_1: ABPC, R_2: BPC, and R_3: MCIPC, divalent PCs are R_4: CBPC, and R_5: SBPC.

TABLE II. Comparison of the Isotachophoretic Results with Those by the Other Method,[17] for the Reaction Products between PCs and KM, and the Degradation Products of PCs

PCs	PM		PA	
	A	B	A	B
ABPC	71	78	29	22
BPC	36	38	64	62
CBPC	45	40	55	60
MCIPC	39	42	61	58
SBPC	35	34	65	66

PM: the reaction products between PCs and KM. PA: the degradation (hydrolysis) products of PCs. A, the results (%) by Yamana *et al.*[17] were represented by the ratio (PM : PA). B, the ratio of PM : PA were calculated from each of the quantitative results by isotachophoresis.

TABLE III. Some Values for the Reaction Products between PCs and KM, and the Degradation of PCs

PCs	PA			PM		
	$h_R \pm$S.D.[a] ($N=5$)	V_A[b]	$\|Z\|$[c]	$h_R \pm$S.D.[a] ($N=5$)	V_A[b]	$\|Z\|$[c]
ABPC	2.15±0.05	304.93	2	5.98±0.08	707.57	1
BPC	1.90±0.08	293.16	2	6.93±0.22	695.80	1
CBPC	1.42±0.10	313.00	3	4.32±0.07	715.64	2
MCIPC	2.19±0.09	341.79	2	6.65±0.21	744.43	1
SBPC	1.42±0.07	331.96	3	4.37±0.24	734.60	2

PA: the degradation (hydrolysis) products of PCs. PM: the reaction products between PCs and KM. *a*) The experimental values. h_R is the relative step height: $h_R = h_S/h_L$,[14] where h_S is the step height of the sample zone, and h_L is that of the leading zone. *b*) The molecular volumes (V_A: Å^3/molecule) were calculated from the data for some atoms or groups.[1] *c*) The electric charge (Z) was estimated from those of PCs in Table I and Fig. 1.

The optimum pH of electrolytes for the estimation of the molecular volume or for the separation of the reaction products between PCs and aminoglycoside antibiotics, PCs, and the degradation products of PCs may be determined from the $h_R - pH_L$ curves[14,15] obtained on the basis of this equation.

Figure 2 shows the relationship between the molecular volume and the relative step height of the reaction products between PCs and KM (PM), PCs and the degradation products of PCs (PA).

The open circles show monovalent PCs ($|Z|=1$) and the closed circles show divalent PCs ($|Z|=2$). As Fig. 1 shows the molecular volumes are almost the same. Therefore, the h_R values of the divalent PCs are about half those of the monovalent PCs.

The open triangles show the hydrolysis products of the monovalent PCs. These are divalents ($|Z|=2$), as Fig. 1 shows, and these molecular volumes are almost the same as those of the PCs. Therefore, the h_R values of these are about half those of the monovalent PCs. The closed triangles show the hydrolysis products of the divalent PCs. These are trivalents ($|Z|=3$), as can be seen in Fig. 1, and these molecular volumes are almost the same as those of the PCs. Therefore, the h_R values of these are about one third those of the monovalent PCs.

The open squares show the reaction products between

TABLE IV. Some Values for the Reaction Products between Cephems and KM, Cephems, and the Degradation of Cephems

Cephems	CA			Cephem			CM		
	$h_R \pm$S.D.[a] ($N=5$)	V_A[b]	$\|Z\|$[c]	$h_R \pm$S.D.[a] ($N=5$)	V_A[b]	$\|Z\|$[c]	$h_R \pm$S.D.[a] ($N=5$)	V_A[b]	$\|Z\|$[c]
CET	1.63±0.07	321.01	2	3.57±0.09	308.64	1	5.70±0.11	723.65	1
CEPR	1.62±0.03	341.58	2	3.65±0.12	329.21	1	5.94±0.42	744.22	1
CTX	1.67±0.03	356.38	2	4.02±0.10	344.01	1	5.45±0.19	759.02	1
CMZ	1.75±0.07	366.50	2	3.90±0.10	354.13	1	6.48±0.24	769.14	1

CA: the degradation (hydrolysis) products of cephems. CM: the reaction products between cephems and KM. *a*) The experimental values. h_R is the relative step height: $h_R = h_S/h_L$,[14] where h_S is the step height of the sample zone, and h_L is that of the leading zone. *b*) The molecular volumes (V_A: Å^3/molecule) were calculated from the data for some atoms or groups.[1] The V_A values of cephems were taken from the previous paper.[1] *c*) The electric charge (Z) of cephems was calculated from the equation: $Z = -1/2\,\Sigma\,[1+\tan h\{2.303/2\,(\mathrm{pH}-\mathrm{p}K)\}]$,[16] and those of CA and CM were estimated from those of cephems and Fig. 1.

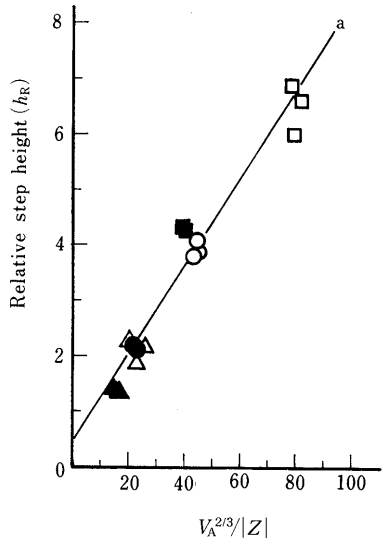

Fig. 2. Relationship between the Molecular Volumes (V_A) and the Relative Step Height (h_R) of the Reaction Products between PCs and KM, PCs, and the Degradation Products of PCs

□, the reaction products between monovalent PCs and KM; ■, the reaction products between divalent PCs and KM; ○, monovalent PCs; ●, divalent PCs; △, the degradation (hydrolysis) products of monovalent PCs; ▲, the degradation (hydrolysis) products of divalent PCs. a) the correlative equation between the V_A and h_R for the reaction products between PCs and KM ($N=5$), PCs ($N=5$), and the degradation products of PCs ($N=5$): $h_R = 0.32 + 0.080 V_A^{2/3}/|Z|$ (Eq. 3, $N=15$, $r=0.972$), where Z is the electric charge.

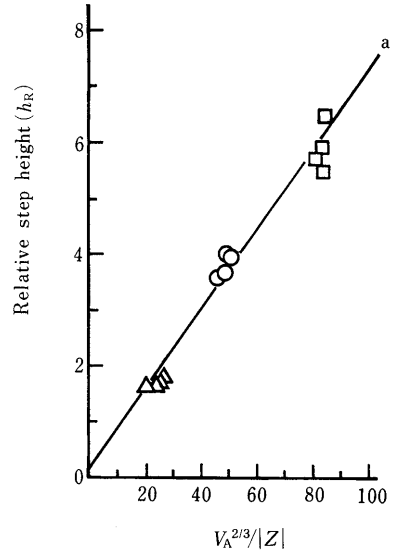

Fig. 3. Relationship between the Molecular Volumes (V_A) and the Relative Step Height (h_R) of the Reaction Products between Cephems and KM, Cephems, and the Degradation Products of Cephems

□, the reaction products between monovalent cephems and KM; ○, cephems; △, the degradation (hydrolysis) products of cephems, a) the correlative equation between the V_A and h_R for the reaction products between cephems and KM ($N=4$), cephems ($N=4$), and the degradation products of cephems ($N=4$): $h_R = 0.04 + 0.072 V_A^{2/3}/|Z|$ (Eq. 4, $N=12$, $r=0.987$), where Z is the electric charge.

the monovalent PCs and KM. These are monovalents ($|Z|=1$), as Fig. 1 shows, and these molecular volumes are about twice those of the PCs. Therefore, the h_R values of these are about twice those of the monovalent PCs. The closed squares show the reaction products between the divalent PCs and KM. These and divalents ($|Z|=2$), as can be seen in Fig. 1, and these molecular volumes are about twice those of the PCs. Therefore, the h_R values of these are almost the same as those of the monovalent PCs.

Consequently, Fig. 2 shows that the molecular volumes of the reaction products between the PCs and aminoglycoside antibiotics, and those of the degradation products of the PCs can be estimated by the relative step height in the isotachopherogram.

(4) Derivation of the Correlation Equation between Molecular Volume (V_A) and the Relative Step Height (h_R) for the Reaction Products between Cephems and KM, Cephems, and the Degradation Products of Cephems The values of V_A, h_R and electric charge (Z) for the reaction products between cephems and KM, and the degradation products of cephems are listed in Table IV.

The following correlative equation between V_A and h_R for the reaction products between cephems and KM ($N=4$), cephems ($N=4$), and the degradation products of cephems ($N=4$) was derived ($p<0.01$).

$$h_R = 0.04 + 0.072 V_A^{2/3}/|Z| \quad (r=0.987,\ N=12) \tag{4}$$

According to this equation, the molecular volume of the reaction products between cephems and the other aminoglycoside antibiotics may be estimated from the value of h_R, when the electric charge is known.

The optimum pH of the electrolytes for estimation of the molecular volume or for separation of the reaction products between cephems and aminoglycoside antibiotics may be determined from the $h_R - \mathrm{pH_L}$ curves[14,15] obtained on the basis of this equation.

Penicillins consist of a five-membered ring and a β-lactam ring, as Fig. 1 shows. On the other hand, cephems consist of a six-membered ring and a β-lactam ring. And, the amino group of kanamycin reacts with the β-lactam ring of the penicillins and the cephems, and hydrolysis occurs in the β-lactam rings, also. Therefore, the hydrolysis products of

cephems and the reaction products between cephems and kanamycin also can be seen in Fig. 1.

Figure 3 shows the relationship between the molecular volume and the relative step height of the reaction products between cephems and KM (CM), cephems and the degradation products of cephems (CA).

The degradation products of cephems are the hydrolysis products of cephems ($|Z|=1$). Therefore, these are divalents ($|Z|=2$), as can be seen in Fig. 1, and these molecular volumes are almost the same as those of cephems. Therefore, the h_R values of the divalent are about half those of cephems.

The reaction products between cephems and KM are monovalents ($|Z|=1$), as can be seen in Fig. 1. These molecular volumes are about twice those of cephems, therefore, the h_R values are about twice those of cephems.

Consequently, Fig. 3 shows that the molecular volumes of the reaction products between cephems and aminoglycoside antibiotics, and those of the degradation products of cephems can be estimated by the relative step height in the isotachopherogram.

As Fig. 1 shows, a molecule of aminoglycoside antibiotics reacts with a molecule of β-lactam antibiotics.[20] And, Fig. 2 and Fig. 3 show that the reactions occur in both cases of penicillins and cephems. The dosage of most of the aminoglycosides is about 10% the dosage of β-lactam antibiotics, clinically.[12,13] Therefore, the inactivation of aminoglycoside antibiotics by interaction is much greater than for β-lactam antibiotics.[10] For these reasons, use of aminoglycosides and β-lactam antibiotics at the same time should be avoided, and the usage time of aminoglycosides should differ from that of β-lactam antibiotics.

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References

1) O. Fujishita, M. Hirakawa, K. Nakashima, S. Higuchi, K. Otsubo, T. Aoyama, and H. Karasawa, *Chem. Pharm. Bull.*, **36**, 3985 (1988).
2) T. Hirokawa, M. Nishino, N. Aoki, Y. Kiso, Y. Sawamoto, T. Yagi, and J. Akiyama, *J. Chromatogr.*, **271**, D1 (1983).
3) O. Fujishita, R. Fukuoka, M. Hirakawa, S. Higuchi, T. Aoyama, and M. Horioka, *Chem. Pharm. Bull.*, **34**, 5026 (1986).
4) T. Hirokawa, T. Gojo, and Y. Kiso, *J. Chromatogr.*, **390**, 201 (1987).
5) T. Hirokawa, T. Gojo, and Y. Kiso, *J. Chromatogr.*, **369**, 59 (1986).
6) P. W. Trown, B. Smith, and E. P. Abraham, *Biochem. J.*, **86**, 284 (1963); A. L. Demain, *Lloydia*, **37**, 147 (1974).
7) K. Nakashima, O. Fujishita, M. Hirakawa, S. Higuchi, K. Otsubo, and T. Aoyama, Abstracts of Papers, The 8th Electrophoresis Symposium (Isotacho), Kyoto, December 1988, pp. 37—38.
8) K. Nakashima, O. Fujishita, M. Hirakawa, S. Higuchi, K. Otsubo, and T. Aoyama, Abstracts of Papers, The Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April 1989, p. 199.
9) L. J. Riff and G. G. Jackson, *Arch. Intern. Med.*, **130**, 887 (1972); M. Davies, J. R. Morgan, and C. Anand, *Antimicrob. Agents Chemother.*, **7**, 431 (1975); L. K. Pickering and P. Gearhart, *ibid.*, **15**, 592 (1979); K. A. O'Bey, L. K. Jim, J. P. Gee, and R. M. Johnson, *Am. J. Hosp. Pharm.*, **39**, 1005 (1982).
10) V. D. Gupta and K. P. Stewart, *Am. J. Hosp. Pharm.*, **40**, 1013 (1983).
11) C. B. Smith, P. E. Dans, J. N. Wilfelt, and M. Finland, *J. Infect. Dise.*, **119**, 370 (1969); Y. Yee, B. Farber, and S. Mates, *ibid.*, **154**, 531 (1986); S. Aonuma, F. Ariji, K, Oizumi, and K. Konno, *Tohoku J. Exp. Med.*, **152**, 119 (1987).
12) "Zen-Tei Iyakuhin Yoran," ed. by Osaka-fu Byoin Yakuzaishi-Kai, Yakugyo-Jiho-Sha, Tokyo, 1983, pp. 1220, 1224, 1226, 1230, 1236, 1238, 1242, 1266, 1268.
13) M. Horioka and K. Fukumuro (ed.), "Shin-Kaihatsu Iyakuhin Binran," 3rd ed., Yakugyo-Jiho-Sha, Tokyo, 1983, pp. 1138, 1193, 1212, 1161, 1241, 1255, 1308.
14) O. Fujishita, S. Higuchi, M. Yoshikawa, T. Aoyama, and M. Horioka, *Chem. Pharm. Bull.*, **31**, 2134 (1983).
15) O. Fujishita, R. Fukuoka, M. Hirakawa, S. Higuchi, T. Aoyama, and M. Horioka, *Chem. Pharm. Bull.*, **34**, 1344 (1986).
16) Y. Kiso and E. Falk, *J. Chromatogr.*, **59**, 401 (1971).
17) T. Yamana, Y. Mizukami, F. Ichimura, and K. Yokogawa, *Yakuzaigaku*, **38**, 74 (1978).
18) G. Gomori, *Proc. Soc. Exp. Biol. Med.*, **62**, 33 (1946).
19) T. Haruki and J. Akiyama, *Anal. Lett.*, **6**, 985 (1973); J. Akiyama and T. Mizuno, *J. Chromatogr.*, **119**, 605 (1976).
20) J. L. Henderson, R. E. Polk, and B. J. Kline, *Am. J. Hosp. Pharm.*, **38**, 1167 (1981); W. A. Kehoe, *Hosp. Pharm.*, **21**, 34 (1986).