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An Innovative Classification of, and a New Structure-activity-relationship Approach to Degradation Kinetics of Cephalosporins: An Attempt to Enhance the Therapeutic Activity

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Degradation kinetics of cephalosporins was innovatively classified into six groups according to its mechanism that is based on the attached functional and/or substituent groups. 3-Position plays an important role in the degradation kinetics, and they are classified into three major groups; one with a 3-acetoxy group, another with a 3-deacetoxy group, and the other without a 3-acetoxy group. Each group is further subdivided depending on whether it contains 7-x-amino group or not. The order of the alkaline hydrolysis of cephalosporins coincided with the order of the degree of their antibacterial activities. This provides an evidence to support the hypothesis that the biological activity of β-lactam antibiotics depends upon the reactivity of the β-lactam moiety. At the same time, the compound should be stable, and the stability is often related to the reactivity of 3-position. Combination products of biodegradable cephalosporins and acid-stable cephalosporins are desired products. An example of such products produced by chemical modifications stated above will be the one with a good leaving group at the 3-position that is not hydrolyzed.

Within the last fifty years the discovery and the development of cephalosporins have made a great contribution to the antibiotic chemotherapy in clinical medicine. There are currently over twenty cephalosporin antibiotics available commercially; among them are cephaloridine, cephalexin, cephaloglycine, cephazolin, cephapirin, cephacetrile, cepharadine and so on. They can be classified into three major groups from the standpoint of degradation kinetics: (I) 3-acetoxycephalosporins such as cephalothin, cephapirin and cephaloglycine; (II) 3-deacetoxycephalosporins such as cephalexin and cepharadine; and (III) 3-non-acetoxycephalosporins, which have 3-acetoxy group replaced by other substituents, such as cephaloridine and cephazolin.[1~8] Each group can be further subdivided into two categories depending upon whether it has an α-amino moiety at 7-position or not (type a and type b).

This discussion will be conducted group by group focusing on kinetically important functional groups as well as studying degradation mechanisms of each drug.[9~21] If any similarity is observed between some aspects of different cephalosporins, duplicated explanation will be omitted. So far, YAMANA *et al.* is the only group who studied comprehensive and comparative degradation kinetics of most of the cephalosporins (Tables 1, 2, Figs. 1, 2), although other workers reported results of degradation kinetics of specific cephalosporins.[21~24]

New Classification

I.A. 3-Acetoxy with 7-α-Amino Group

A semisynthetic cephalosporin, and cephaloglycine belong to this group (Table 1). Like other 3-acetoxy cephalosporins ester cleavage takes place between pH 2 and 5 at the 3-position to produce an alcohol derivative under

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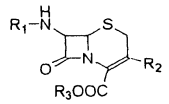
Table 1-a. A reported properties of cephalosporins.



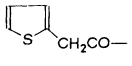
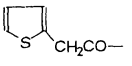
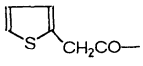
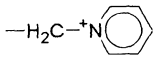
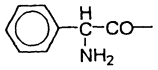
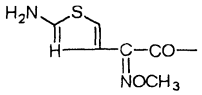
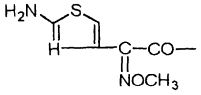
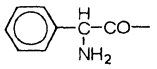
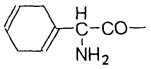
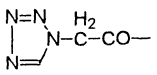
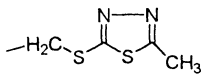
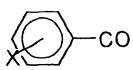
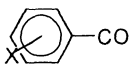
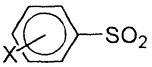
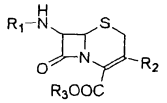
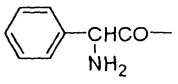
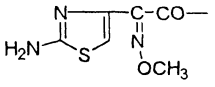
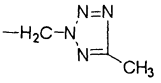
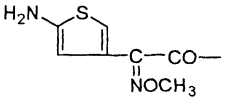
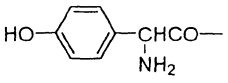
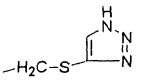
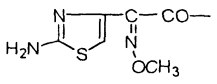
Cephalosporin	substituents R1	R2	R3	analytical method	pKa
Cephalothin		CH2OCOCH3	Na	HPLC, UV, iodometry	pKa= 2.22
Deacetylcephalothin		CH2OH	Na	HPLC	-
Cephaloridine			-	HPLC, UV, iodometry	pKa= 1.67
Cephaloglycin		CH2OCOCH3	H	HPLC, UV, iodometry	pKa= 1.91
Cefotaxime		CH2OCOCH3	H		p Ka=3.4
Desacetylcefotaxime		CH2OH	H	HPLC, UV	-
Caphalexin		CH3	H	HPLC, UV, iodometry hydroxamic acid assay	pKa= 2.56 pKa$_2$=6.88
Cephradine		CH3	H	HPLC, UV, iodometry hydroxamic acid assay	pKa= 2.53 pKa$_2$= 7.30
Cefazolin			Na	UV, iodometry	pKa= 2.54 pKa$_2$= 1.70
Substituted Phenylcephalosporin(1) [a,4-CH3; b,H; c,4-Cl; d, 4-NO2; e,3,5-(NO2)2]		CH2OCOCH3	Na	UV, idometry	
Substituted phenyl-Deacetpxycephalosporin (II)(a,4-CH3; b,H; c,4-Cl; d,4-NO2)		CH3	Na	UV, idometry hydroxamic acid assay	
7-Benzenesulfonamido-cephalosporin(III)		CH3	Na	UV, idometry	
7-Aminocephalosporanic acid	H	CH2OCOCH3	Na	UV, idometry	pKa=2.02 pKa$_2$=4.42
7-Aminodeacetoxy-cephalosporanic acid	H	CH3	H	UV, idometry hydroxamic acid assay	pKa=2.95 pKa$_2$=4.87

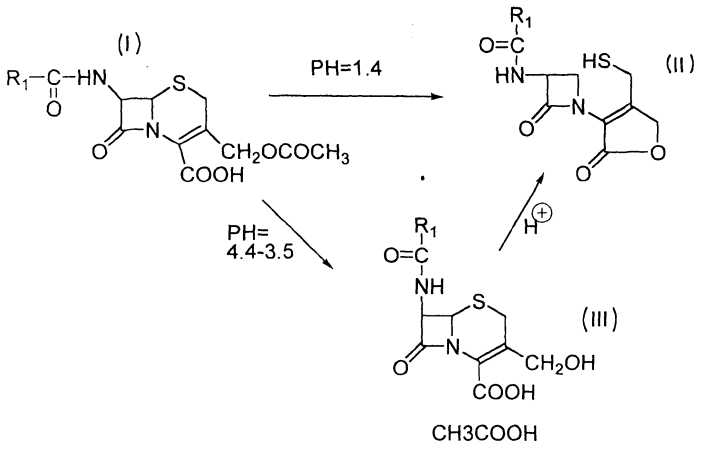
Table 1-b. Reported properties of cephalosporins.



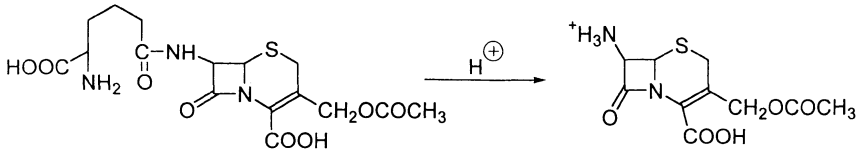
Cephalosporin	substituents R1	R2	R3	analytical method	pKa
Cefaclor		Cl	H		pKa=1.56, 7.22
Cefteram			H		pKa=3.0
Cefpodoxime		CH_2OCH_3	H		pKa=3.20±0.13
Cefatrizine			H		
Cefetamet		CH3	H		

Cited in part from reference (24) with the permission from the author.

Scheme 1.



Scheme 2.

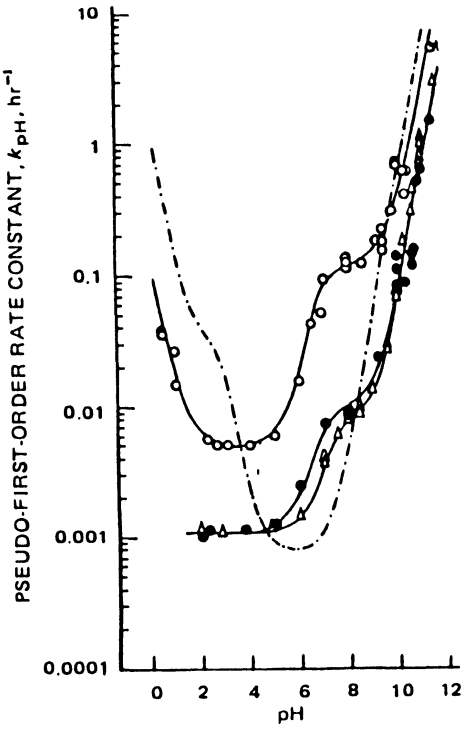


specific acid catalysis (Scheme 1).[22] At pH 2 or below a more unique compound, lactone [II] is formed. The compound [III] can undergo lactonization with the further aid of hydrogen ions.[23]

Besides the 3-acetoxy group, the β-lactam ring is subject to hydrolysis in the acidic media. YAMANA *et al.*[24] found that regardless of the side chain R (Table 1), the rate of hydrolysis of the β-lactam ring in acidic media is almost the same for all 3-acetoxycephalosporins. Therefore, the degradation mechanism must be more simple than thought, specific hydrogen-ion-catalyzed hydrolysis rather than acid-catalyzed intramolecular attack of the side-chain amino on the β-lactam ring. β-Lactam cleavage in acidic media is comparatively slower than the hydrolysis of a functional group at 3-position. The 8-fold difference in magnitude was observed between them as comparison.[24]

The third position in the structure where acid hydrolysis could take place is an amide at 7-position common to all therapeutically useful cephalosporins. LODER *et al.*[25] subjected cephalosporin C to mild acid hydrolysis and obtained low yields of 7-aminocephalosporanic acid (7-ACA) (Scheme 2). The proposed mechanism for the formation of 7-ACA involved diazotization of the amino function on the side chain to give (12) which then reacts intramolecularly to produce the iminolactone (13). 7-ACA is then formed from the solvolysis of (13). This reaction is intramolecular in nature and needs amino function at the end of the side chain to lead to diazotization. Any of the seven commercially available cephalosporins in this grouping do not contain this amino function at the end of the side chain. Therefore, this reaction may not take place for 7-position-amide cephalosporins. Actually there is no report on the hydrolysis of 7-side chain. This is a candidate for hydrolysis without the acid of amino group, although hydrolysis of amide is several times slower than that of ester hydrolysis. At this point all one can do is to postulate that a careful study may find some products from the hydrolysis of 7-amide functional group at very low pH.

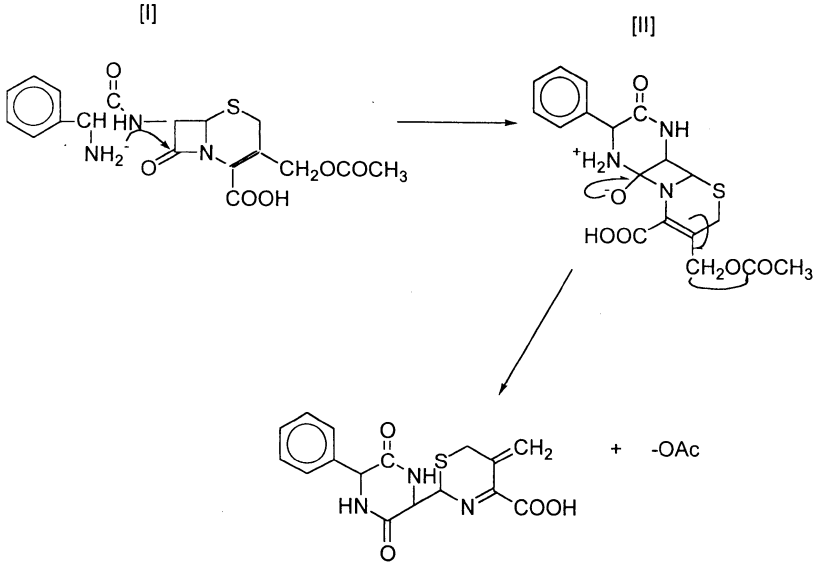
Fig. 1. $-\text{Log } k_{pH}$-pH profiles for the degradation of cephaloglycin (○), cephalexin (●), and cephradine (△) at 35° and $\mu=0.5$.



The dashed-dotted line refers to the pH-rate profile for ampicillin at 35° and $\mu=0.5$ (27).

In the region of pH 5 or above we observe the rate increase (Fig. 1) and there is an inflection point at around pH 7 which corresponds to the pKa 2 of α-amino group. Therefore, intramolecular nucleophilic attack of the α-amino on the carbonyl carbon of β-lactam was proposed.[24,26] Scheme 3 shows that proposed mechanism. This degradation pathway can be applied to all of type (a) cephalosporins, which contain α-amino group, such as

Scheme 3.



cephalexin and cepharadine as well as cephaloglycine.

The unit slope observed in the region of pH 10 or above represents the specific hydroxide-ion-catalyzed degradation. Consequently, overall rate expression can be formulated as:

$$k_{pH}=k_H a_H+k_o+k_b[K_{a2}/(K_{a2}+a_H)]+k_{OH}(K_w/a_H) \quad (1)^{24)}$$

where k_{pH} is the apparent first order rate constant, and k_b is the spontaneous degradation rate constant of the anionic species of cephaloglycine.

As a result of the buffer used, English workers[27] found a displacement reaction of acetyl group to produce a new antibiotic that possessed no net charge at pH 7 (1). This leads to the more biologically active cephalosporins such as cephaloridine that could be converted from cephalothin (Scheme 4).[28,29] This displacement reaction occurs by an SN1 mechanism.[29] A rate determining ionization takes place first and then the ion (2) is added to the nucleophile.

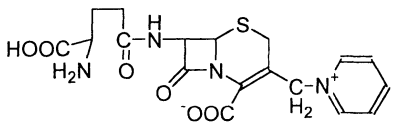
Common to all 3-acetylcephalosporins (α-amino, or no-α-amino), specific base-catalyzed hydrolysis seems to take place in one of two ways. One is the usual ester hydrolysis at 3-position. The other hydrolysis results from an attack of base on the 8-carbonyl carbon as shown in Scheme 5.[30,31] This mechanism was supported by other workers[32,33] who proved the existence of an intermediate analogous to (3). KONECNY *et al.*[34] calculated the magnitude of the rate differenced between these two types of specific base catalyzed hydrolysis and found that the ester hydrolysis at 3-position is about four times as fast as that of the β-lactam ring opening.[35]

I.B. 3-Acetoxy without 7-α-Amino Group

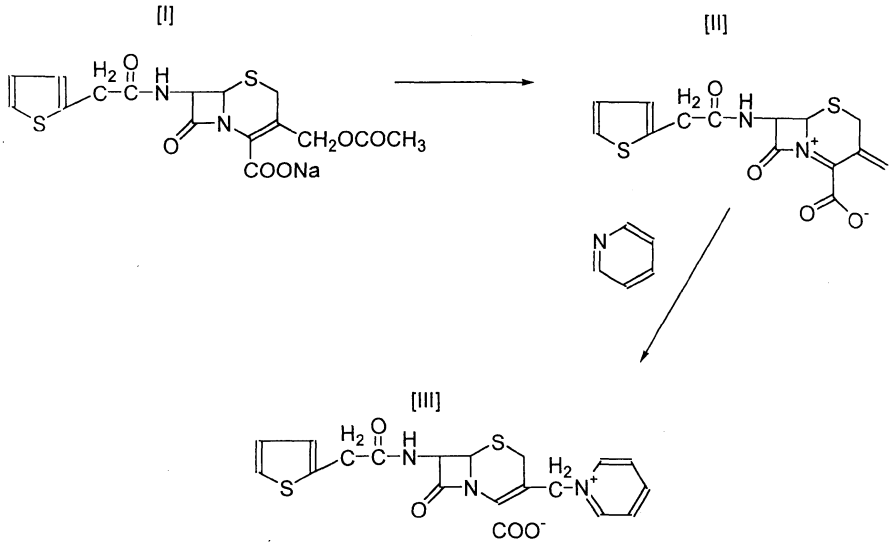
Cephapirin, cephalothin, and cefotaxime, which belong to this group, do not possess an acyl amine function at 7-position. Therefore, they do not exhibit good antibiotic activity.[14] However, the antibiotics of this group aroused our interest to study the degradation kinetics of cephalosporins. This group features the very interesting long-range plateau extending from pH 3 to pH 8. YAMANA *et al.*[24] carried out a kinetic study using substituted phenoxycephalosporin (4) and showed that election donating substituents increased the rate of β-lactam cleavage, and supported a previously reported mechanism (Scheme 6).[36] JEFFERY *et al.*, on the other hand, isolated a small amount of thiazole (7) by warming cephalosporin C in aqueous solution at pH 7 and argued that sulfur attacks on the acyl carbon of the side chain (Scheme 6′). They postulated that fission of sulphur-carbon bond is fairly strong and it is not likely to undergo fission as the initial step in the neutral pH. Rather, we believe that the intramolecular β-lactam cleavage takes place first and then a small portion of it can be converted to thiazole due to the intramolecular attack of sulfur on the carbonyl carbon. Therefore, YAMANA's theory based on kinetics data seems to be more realistic.

To prove that water is not a catalyst in this plateau

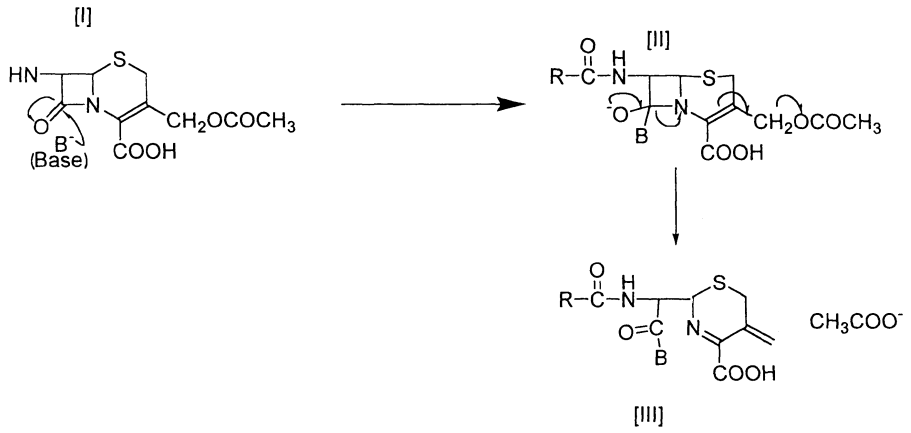
Scheme 4.



(1)

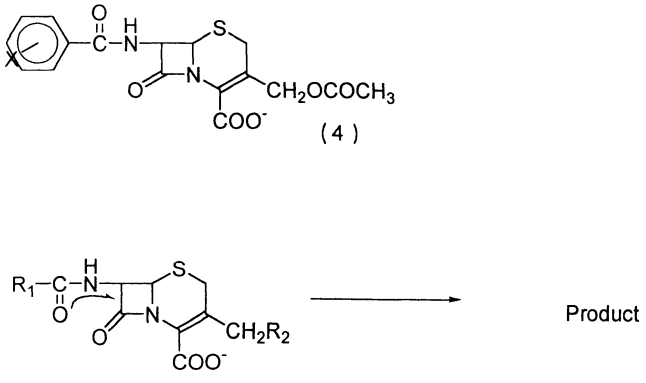


Scheme 5.

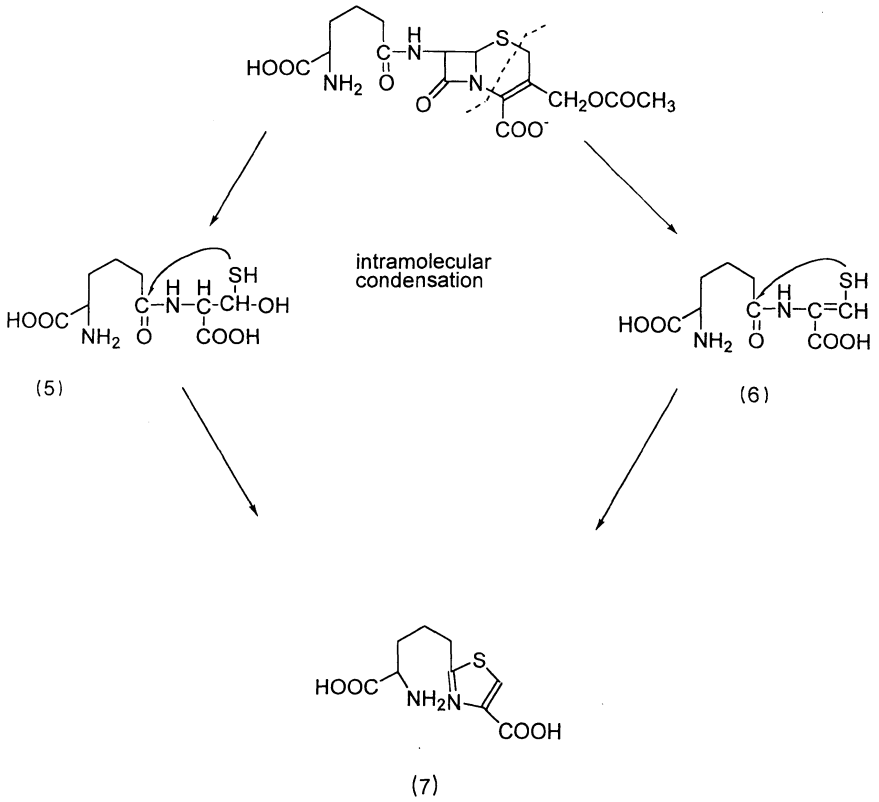


region, YAMANA *et al.* performed an experiment on the deuterium solvent isotope effect and obtained the value of $KoH_2O/KoD_2O=0.93$. This is good evidence that water does not play a significant role in the initial opening of the β-lactam ring. This also disproves JEFFERY *et al.*'s aforementioned sulfur-carbon fission theory in which they

Scheme 6.



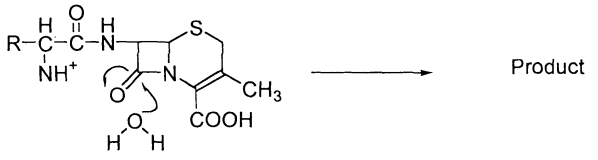
Scheme 6′.



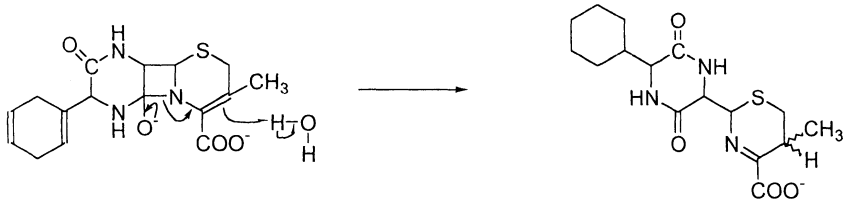
stated that hydrolytic changes are involved. As shown in Fig. 2, the degradation of this compound is not influenced by the dissociation of 4-carboxylic acid group (pKa=2.22). This finding makes an interesting contrast to all types of penicillins whose degradation is influenced by 3-carboxylic acid group.

A fairly new semisynthetic cephalosporin, cephapirin,[37,38] belongs to this category (13), but the degradation kinetics has not yet been reported. From the fact that this compound and cephalothin have the same kinetically important functional group, we may reasonably predict a similar degradation profile. The solubility *versus* pH 5 plot has been reported previously[37,38] and the shape resembles the pH rate profile of cephalothin. Decrease in the solubility between pH 2 and pH 5 is an indication of the presence of zwitterions. The pKa values of cephapirin are reported to be 2.03 and 5.35.[38] As shown in Fig. 2 pH rate profile, 7-ACA shows a slight rate increase in the plateau

Scheme 7.



Scheme 8.



(8)

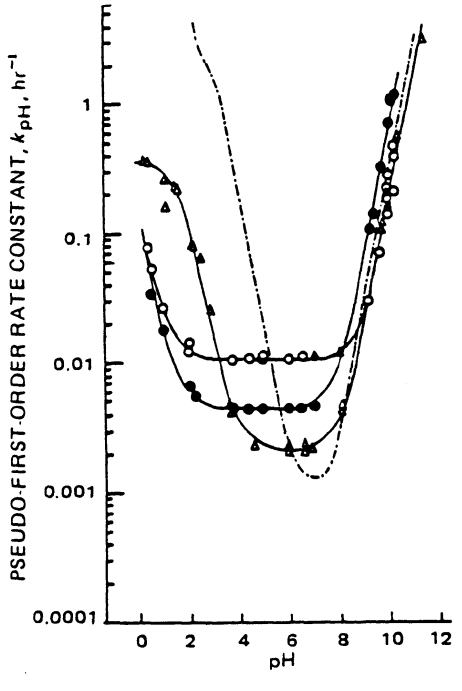
region. This rate increase may be attributed to the inductive effect of protonated amino group at 7-position. The pKa of the amino group is 4.42 and the inflection point was observed at around that pH.

II. 3-Deacetoxy, 7-α-Amino Group

Cephalexin, and cephradine, desacetylcehalothin, desacetylcefotaxime, cefpodoxime, and cefetamet belong to this group. These two compounds show almost identical pH-rate profile as shown in Fig. 1. Below pH 5, water-catalyzed cleavage of the β-lactam ring takes place as shown in Scheme 7. This pH independency indicates that if the compound is protonated completely the hydrogen-ion-catalyzed reaction is negligible.

As was discussed under cephaloglycine, α-amino-characterized β-lactam cleavage takes place above pH 5. But there is a slight difference in the final stage of the degradation products between the two cases due to the different types of 3-substituents. From the mass data along with the NMR data, COHEN *et al.*[39] confirmed the existence of alkaline degradation product (8) of cephradin. This is to add to the postulate of HAMILTON-MILLER *et al.* which reported several aminolysis products which did not include compounds analogous to (8). Reported compounds (9) by HAMILTON-MILLER *et al.* retain $\Delta 3$ position, and he did not give the conclusive evidence to prove the existence of this compound. In this case nitrogen at 5-position picks up a

Fig. 2. $-\text{Log } k_{pH}$-pH profiles for the degradation of cephalothin (○, in H_2O; ▲, in D_2O), cephaloridine (●), and cefazolin (△) at 35° and $\mu=0.5$.



The dashed-dotted line refers to the pH-rate profile for penicillin G at 35° and $\mu=0.5$ (28).

Note: Cited from reference 24 with the permission from the author.

proton from the solvent without shifting the double bond on the ring, whereas in compound (8) carbon at 3-position picks up a proton from the solvent (Scheme 8). From the fact that the double bond in the ring shifts fairly easily, it seems that the majority of the products exist as a form of compound (8).

III. 3-Non-acetoxy Group

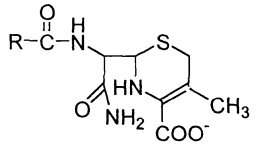
In this group an acetoxy group is replaced by other nucleophiles such as pyridines,[40,41] other aromatic heterocycle,[42] xanthenes[43] *etc.* Our examples are the most commonly used agents, cephaloridine, cefazolin, cefaclor, cefteram and cefatrizine. The pH-rate profile of these compounds is very similar as shown in Fig. 2. Greater similarity is observed between cephaloridine and cephalothin indicating that acetyl group and the  group as well as the  and the  groups in each side chain present a similar kinetic degradation. Most of the noteworthy discussion has already been made under cephalothin, but the difference in the rates at the plateau region drew our interest very much. This difference may be due to a little bulky  group on cephaloridine which hinders water from getting in, and also may be due to the presence of a better leaving group  over $-OCOCH_3$.[44]

Cefazolin degradation on the other hand has to be interpreted a little differently. In the Plateau region it is more stable than cephalothin and cephaloridine. This maybe due to the poor leaving group ability of thiadiazole that resists its cleavage from the 3-methyl group. In the low pH region we observe an inflection point at around pH 2 which reflects the pKa of thiadiazole ($pK_a = 1,7$). Therefore, the hydrogen-ion-catalyzed hydrolysis takes place on the thiadiazole moiety and the rate of elimination is much faster than that of cephaloridine.[45]

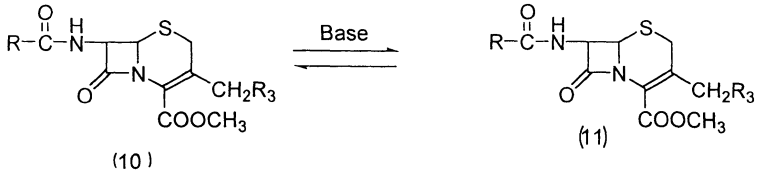
Overall Discussion

As the group-by-group discussion comes to an end, observations will be made on other aspects of degradation

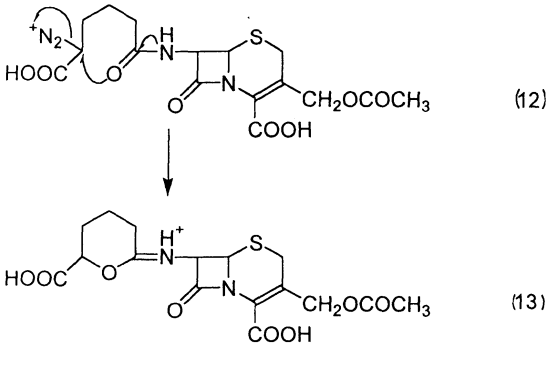
Scheme 8′.



(9)



(10) R_3 =OCOCH
(11) R_3 =H



(12)

(13)

which have not been dealt with in the group-by-group session. Firstly, possible isomerization of 3-cepham double bond ($\Delta 3$) to 2-cepham double bond ($\Delta 2$) is worth mentioning because a $\Delta 2$ cephalosporin is biologically inactive. GREEN *et al.*[46] first reported a slow process of isomerization of cephalosporanic acid in 1965. The more facile double bond isomerization was also reported to occur with amine base when the carboxyl group is esterified or otherwise blocked.[47,48] As shown in Scheme 8′ a 7 : 3 ratio of 2-cephan to 3-cepham isomers occurs for acetoxycephalosporin (10) in basic media. MORIN *et al.*[49] reported that the equilibrium composition between two isomers is dependent on the size of the 3-methyl substituents. When R is H (11), the isomeric composition was found to be 3 : 7 for 2-cephan to 3-cepham (Scheme 8′). This ratio is the opposite to that of acetoxycephalosporins. Since the carboxyl group of therapeutically useful cephalosporins is not esterified, this isomerization process is slow. But during the manufacturing process for its synthesis or when finished products are kept in the basic condition for a long time the inactivation due to isomerization could produce a problem.

The next interesting observation is kinetic proof of the theory that the magnitude of biological activity of β-lactam antibiotics is related to the reactivity of the β-lactam moiety toward base.[50~52] The order of the alkaline hydrolysis

Table 2. Various rate constants for the degradation of cephalosporins and penicillins at 35° and μ=0.5.

β-Lactam Antibiotic	$k_H, M^{-1}hr^{-1}$	10^3k_o, hr^{-1}	$10^{-3}k_{OH}$ $M^{-1}hr^{-1}$	10^2k_a, hr^{-1}	10^2k_b, hr^{-1}	10^3k_c, hr^{-1}	10^3k_d, hr^{-1}
Cephalosporins							
Cephalothin	0.172	10.9	10.6				
Cephaloridine	0.134	4.4	38.8				
Cephaloglycin	0.148	5	13.1		13.5		
Cephalexin	d	1.15	2.64		1.01		
Cepharadine	d	1.1	3.98		0.74		
Cefazolin	d	2.15	11.4	37			
Ia	0.238	21	9.08				
Ib	0.206	18.7	8.56				
Ic	0.186	11.4	8.45				
Id	0.265	7.06	9.55				
Ie	0.176	6.56	8.85				
IIa	e	0.27	1.43				
IIb	e	e	1.33				
IIc	e	0.27	1.56				
IId	e	e	1.52				
III	e	3.3					
7-Amino cephalosporanic acid	0.579		2.32			10.4	6
7-Aminodeacetoxy cephalosoiranic acid	d		0.36			1.84	0.28
Penicillins.f							
Penicillin G	601	0.9	11.9	341			
Carbenicillin	52.2	2.04	12.1	183			
Cloxacillin	35.6	0.94	13.4	21			
Propocillin	30.7	0.89	17.3	13.3			
Cyclacillin	4.61	2.49	11	4.33			
Ampicillin	1.82	0.75	25.7	5.56			
Substituted phenylpenillins							
4-CH3	e	e	17	2188			
H	e	e	e	977			
4-Cl	e	e	18.3	399			
3,4-(Cl)2	301	1.45	16.2	104			
4-(NO2)2	127	1.45	17.9	49.8			
3,5-(NO2)2	15	1.55	17.4	5.98			
6-Aminopenicillanic	0.608		3.15			11.4	3.03

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Table 3. Antimicrobial activity and route of administration of selected cepharosporins.[54~66]

Cephalosporin	MIC (μg/mL)	MIC Tested strain	oral or parenteral	Gram-negative or positive
Cephalothin	1.56-6.25	Escherichia coli	parenteral	both
Cephaloridine	4	Escherichia coli	parenteral	both
Cephaloglycin	1->128(2)*	Escherichia coli	oral	-
Desacetylcephaloglycin	4-128(8)*	Escherichia coli	-	-
Cefotaxime	0,125	Escherichia coli	parenteral	both
Desacetylcefotaxime	0,78	Escherichia coli	-	-
Cephalexin	6.25-12.5	Escherichia coli	oral	both
Cephradine	$\leqq$ 1	Sensitive	oral	both
Cefazolin	$\leqq$ 8	average of various strains	parenteral	both
Cefaclor	2	Escherichia coli	oral	both
Cefteram	0.05-0.39	Escherichia coli	oral	both
Cefpodoxime	0.5	Escherichia coli	oral	negative
Cefatrizine	0.78-3.13	Escherichia coli	oral	both
Cefetamet	0.39	Escherichia coli	oral	both
Cephapirin	0.01-500	average of various strains	oral	negative
Cefprozil	8	most (90%) strains	oral	both

*MIC value for most strains

of cephalosporin is cephaloridide>cephaloglycine> cephalothin>cephalexin (Table 2), and this order roughly coincides with the order of the degree of their antibacterial activities although MIC depends on types of strains tested (Table 3). On the other hand, we need to produce stable antibiotics especially at physiological pH and acidic pH if orally used. Cephalexin shows its remarkable acid stability but also shows fairly high alkaline stability that correlates to the inferior antibiotic activity to other cephalosporins. Therefore, a combination product of cephaloridine and cephalothin will make a very interesting antibiotic. The rate of hydrolysis of β-lactam seems to be correlated to the leaving group ability attached on the carbon at 3-position whose substituent is cleaved after carbonyl carbon at 8-position is attacked by base. On the other hand, the major rate of hydrolysis in the acidic region is correlated to the cleavage of the ester at 3-position. Hence, attachment at 3-position of a good leaving group which is not hydrolyzed make a drug which is acid stable and is readily hydrolyzed in the basic condition.[53] Such a drug if it is formulated may improve pharmaceutical and therapeutic properties of cephalosporins to some extent. The authors sincerely hope that some readers of this article develop such new cephalosporins based on our theory.

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