peptide can be accounted for by interaction with no more than four or five amino acid residues. In Leu-enkephalin, for example, the observed binding can be explained solely in terms of the tyrosine and phenylalanine residues, in complete accord with the conclusions of structure–activity data.[18,19]

Registry No. 1, 50-53-3; 2, 50-48-6; 3, 1668-19-5; 4, 438-60-8; 5, 91-84-9; 6, 113-92-8; 7, 486-12-4; 8, 486-16-8; 9, 5786-21-0; 10, 24219-97-4; 11, 50-47-5; 12, 53179-07-0; 13, 613-67-2; 14, 835-31-4; 15, 1491-59-4; 16, 50-60-2; 17, 51-41-2; 18, 59-39-2; 19, 4205-90-7; 20, 66711-21-5; 21, 59803-99-5; 22, 31036-80-3; 23, 31428-61-2; 24, 76280-95-0; 25, 28125-87-3; 26, 5051-62-7; 27, 24248-22-4; 28, 29110-47-2; 29, 7361-61-7; 30, 146-48-5; 31, 7762-32-5; 32, 19216-56-9; 33, 26844-12-2; 34, 525-66-6; 35, 26839-75-8; 36, 13655-52-2; 37, 60106-89-0; 38, 749-02-0; 39, 2062-78-4; 40, 52-86-8; 41, 57808-66-9; 42, 74050-98-9; 43, 15574-96-6; 44, 60085-78-1; 45, 129-03-3; 46, 17692-51-2; 47, 361-37-5; 48, 1893-33-0; 49, 2062-84-2; 50, 1841-19-6; 51, 26864-56-2; 52, 68844-77-9; 53, 24526-64-5; 54, 487-93-4; 55, 50-67-9; 56, 61-54-1; 57, 54-04-6; 58, 50-37-3; 59, 58-00-4; 60, 2709-56-0; 61, 51-61-6; 62, 56-12-2; 63, 2763-96-4; 64, 439-14-5; 65, 846-49-1; 66, 1812-30-2; 67, 115-38-8; 68, 50-11-3; 69, 2964-06-9; 70, 22173-64-4; 71, 17617-45-7; 72, 2571-22-4; 73, 57-41-0; 74, 298-46-4; 75, 144-62-7; 76, 884-33-3; 77, 501-52-0; 78, 55700-98-6; 79, 75-39-8; 80, 64-19-7; 81, 75-04-7; 83, 18771-50-1; 84, 32017-56-4; 85, 5699-58-1; 86, 51528-59-7; 87, 13147-57-4; 88, 35752-42-2; 89, 75521-69-6; 90, 27442-42-8; 91, 51321-79-0; 92, 60949-21-5; 93, 11033-22-0; 94, 92315-28-1; 95, 13484-63-4; 96, 92315-29-2; 97, 63250-34-0; 98, 315-30-0; 99, 2465-59-0; 100, 59708-52-0; 101, 53758-22-8; 102, 56030-54-7; 103, 59708-47-3; 104, 6440-26-2; 105, 357-56-2; 106, 76-99-3; 107, 4310-87-6; 108, 469-79-4; 109, 14521-96-1; 110, 52485-79-7; 111, 36292-69-0; 112, 58239-89-7; 113, 51395-54-1; 114, 51583-02-9; 115, 58-14-0; 116, 59-05-2; 117, 6015-76-5; 118, 70997-40-9; 119, 47035-30-3; 120, 7761-45-7; 121, 738-70-5; 122, 138-81-8; 123, 60698-89-7; 124, 58-85-5; 125, 73-32-5; 126, 96-15-1; 127, 64-04-0; 128, 300-62-9; 129, 16088-07-6; 130, 5241-58-7; 131, 4754-39-6; 132, 92418-74-1; 133, 52-52-8; 134, 4385-91-5; 135, 108-95-2; 136, 51-55-8; 137, 63-75-2; 138, 86-13-5; 139, 51-34-3; 140, 50-52-2; 141, 92-13-7; 142, 21888-98-2; 143, 70-22-4; 144, 485-35-8; 145, 54-11-5; 146, 51-84-3; 147, 51-83-2; 148, 363-24-6; 149, 745-65-3; 150, 551-11-1; 151, 77-92-9; 152, 97-67-6; 153, 528-44-9; 154, 57-83-0; 155, 521-18-6; 156, 50-28-2; 157, 50-02-2; 158, 71-58-9; 159, 521-18-6; 160, 50-22-6; 161, 52-39-1; 162, 10540-29-1; 166, 19993-20-5; 167, 488-69-7; 168, 643-13-0; 169, 63-37-6; 170, 14265-44-2; 171, 76-22-2; 172, 50-99-7; 173, 390-64-7; 174, 298-57-7; 175, 630-60-4; 176, 38838-26-5; 177, 52978-30-0; 178, 51-24-1; 179, 2055-97-2; 180, 67-30-1; 181, 51-48-9; 182, 34645-84-6; 183, 77182-38-8; 184, 15307-86-5; 185, 15687-27-1; 186, 22204-53-1; 187, 5104-49-4; 188, 40013-87-4; 189, 17413-79-5; 190, 99-66-1; 191, 66-76-2; 192, 81-81-2; 193, 15074-17-6; 194, 50-29-3; 195, 60-57-1; 196, 58-89-9; 197, 63-25-2; 198, 1563-66-2; 199, 50-33-9; 200, 51-92-3; morphine, 57-27-2; butaclamol, 51152-91-1; desmethyldiazepam, 1088-11-5; glycine, 56-40-6; leucine, 61-90-5; serine, 56-45-1; tyrosine, 60-18-4; glutamic acid, 56-85-9; lysine, 56-40-6.

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3-Substituent Effect and 3-Methylene Substituent Effect[1] on the Structure–Reactivity Relationship of 7β-(Acylamino)-3-cephem-4-carboxylic Acid Derivatives Studied by Carbon-13 and IR Spectroscopies[2]

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Relationships between the chemical reactivity of 3-substituted cephalosporins or 3-methylene-substituted cephalosporins and several parameters observed by ^{13}C NMR and IR spectroscopies are described. Among 3-substituted cephalosporins, the values of δ(C-3) and δ(COO) of ^{13}C NMR spectra are correlated with the logarithms of the rate constants for alkaline hydrolysis (log k_{obsd}) when substituents at the 3-position are classified into two groups, i.e., OR substituents and others. Among the 3-methylene-substituted cephalosporins, the difference values of the ^{13}C chemical shifts for C-3 and C-4, $\Delta\delta$(4-3), are correlated with log k_{obsd}. The β-lactam $\nu_{C=O}$ value of the solution IR spectra is a good index for the prediction of a significant change of the β-lactam reactivity resulting from modification of a 3-substituent or a 3-methylene substituent. From analysis of these observed parameters, both resonance and inductive effects of the substituent at the 3-position were found to affect the chemical reactivity of the β-lactam ring in cephalosporin, while only the inductive effect of the substituent at the 3′-position was found to affect the β-lactam reactivity.

β-Lactam antibiotics, such as penicillins, cephalosporins, and oxacephalosporins, inhibit biosynthesis of bacterial cell walls by acylating and thereby inactivating transpeptidases and carboxypeptidases.[3] Because the antibacterial activity of an antibiotic depends on the acylation of those enzymes by the β-lactam ring of the antibiotic, the chemical reactivity that represents the acylating ability of the β-lactam ring is an important factor affecting the antibacterial activity. Thus, much interest has been attached to investigation of the structure–reactivity relationship of cephalosporins and penicillins as the first stage in the prediction of antibacterial activity.

A number of parameters have been proposed as indicators of the β-lactam reactivity, for example, the IR

† Deceased.

(1) Cephalosporins were examined from two viewpoints according to the structure of the substituent at the 3-position, i.e., one with the substituent at the 3-position (called the 3-substituted cephalosporin in this paper) and the other with a methylene group at the 3-position (called 3-methylene-substituted cephalosporin in this paper). Here, we define 3-methylene substituent as a group at the 3′-position of a cephalosporin (i.e., the substituent is on the methylene at the 3-position) and a 3-substituent as a whole group, e.g., CH_2R or R′, i.e., a direct 3-substituent, attached to the 3-position of either a cephalosporin or a direct 3-substituted cephalosporin, respectively.
(2) Some part of this study has been reported in a communication: Nishikawa, J.; Tori, K. *J. Antibiot.* 1981, *34*, 1641.

0022-2623/84/1827-1657$01.50/0

carbonyl stretching frequency[4-6] (β-lactam $\nu_{C=O}$), the calculated electron density at the β-lactam carbonyl oxygen[5,7,8] ($Q(O_9)$), and the theoretical transition-state energy[7-11] (TSE), which are calculated by the CNDO/2 method, the C—N and C=O bond lengths, and the distance of the β-lactam nitrogen atom from the plane formed by its three neighboring carbons.[12] Among these parameters, the β-lactam $\nu_{C=O}$ value, the $Q(O_9)$ value, and the TSE value have been known to be excellent indicators of the substituent effect on the β-lactam reactivity when the substituents, such as one on the 3-methylene, do not affect the pyramidal structure of the β-lactam nitrogen atom. We also reported[13] that the difference values of the ^{13}C chemical shifts for C-3 and C-4, $\Delta\delta(4-3)$, show linear correlation with the logarithms of the rate constants, k_{OH}, reported by Yamana and Tsuji[14] for the OH^--catalyzed degradation of cephalosporins, and the $\Delta\delta(4-3)$ value was suggested to be an index for the effect of the 3-methylene substituent on β-lactam reactivity.

The relationships between some parameters and antibacterial activity as well as β-lactam reactivity have been reported. Morin et al.[4] suggested that the β-lactam $\nu_{C=O}$ values show a rough but positive correlation with antibacterial activity. Moreover, Boyd et al.[11] reported that a parabolic relationship between the TSE values and minimum inhibitory concentrations against Gram-negative pathogenic microbes (MICs) exists among various 3-methylene-substituted cephalosporins. As mentioned above, a 3-methylene substituent of cephalosporin has been known to greatly affect the β-lactam reactivity and thereby have an influence on the antibacterial activity.

Some cephalosporins having "direct" substituents at the 3-position (3-R; for example R = Cl, OCH_3, H, etc.) show clinically useful antibacterial activity[15] comparable to that exhibited by 3-methylene-substituted cephalosporins. Thus, Boyd has reported[7] that both the electron density on the β-lactam carbonyl group [for example, the Mulliken overlap population for the β-lactam carbonyl bond $C_8=O_9$, $n(C_8=O_9)$, and $Q(O_9)$] and the TSEs calculated by quantum mechanical treatment for various cephalosporins including those with "direct" substituents at the 3-position can be correlated with alkaline hydrolysis rates.

We also investigated 3-substituent and 3-methylene substituent effects on the reactivity of the β-lactam ring of cephalosporin by using the ^{13}C chemical shift as the first indicator for analysis. In this report, we describe the effect of 3-substituents on the β-lactam reactivity of cephalosporins as well as that of 3-methylene substituents by using the ^{13}C chemical shift, the β-lactam $\nu_{C=O}$ value, and the pseudo-first-order rate constant (k_{obsd}) for hydrolysis of the β-lactam ring, which has been regarded as a model of the reaction of the β-lactam ring with the enzymes.

Experimental Section

^{13}C FT NMR was recorded on a Varian XL-100-12A NMR spectrometer (25.16 MHz) at ordinary probe temperature (31 °C) in 10- and/or 5-mm spinning tubes in $(CD_3)_2SO$ (internal Me_4Si reference, δ 0.0) and D_2O (internal dioxane reference, δ 67.4). The concentrations were fixed between 0.1 mmol/mL and 0.2 mmol/mL because ^{13}C chemical shifts of cephalosporin analogues are influenced slightly by the concentration.[16] ^{13}C spectra of some cephalosporin sodium salts were measured with saturated solutions (ca. 5 mg/mL) because of their lesser solubility. Typical FT NMR measurement parameters were as follows: spectral width, 6016 Hz; pulse width, 7 μs (flipping angle 17°); acquisition time, 0.8 s; number of data points, 9625. ^{13}C NMR signals were assigned by using single-frequency and noise off-resonance decoupling and ^1H nondecoupling with NOE in the gated mode and by comparison of ^{13}C relaxation time T_1[13,17] and of the chemical shifts with those of related compounds.[13,17]

IR spectra were recorded on a JASCO DS-403G grating spectrometer calibrated for the rotational bands of vapor. Cephalosporin esters were dissolved in $CHCl_3$ at ca. 0.0025 M (cell length 0.5 cm) and sodium salts were dissolved under nitrogen stream in dry $(CH_3)_2SO$ at ca. 0.02 M (cell length 0.025 cm). The accuracy of the $\nu_{C=O}$ value was ±1.0 cm^{-1}.

The loss of the UV absorbance at ca. 265 nm of each cephalosporin at pH 10.0 and 35 °C was measured as a function of time with a Hitachi UV 320 automatic recording spectrometer, and the values of the pseudo-first-order rate constants, k_{obsd}, were determined by the method of Guggenheim.[18,19]

MICs were determined by the agar dilution method using sensitivity test agar (Eiken, Japan). An overnight culture of bacteria in tryptosoy broth (Eiken, Japan) was diluted to about 10^6 cells/mL with the same broth. One loopful of this suspension was inoculated with an inoculating device onto agar containing serial twofold dilutions of an antibiotic. Organisms were incubated at 37 °C for 18–20 h. The MIC of an antibiotic was defined as the lowest concentration that inhibited visible growth.

Results and Discussion

The ^{13}C NMR spectra and IR spectra of cephalosporinate ions **1a–9a, 12a, 14a, 15a** and their benzhydryl esters **1b–4b, 6b, 7b, 9b–15b** with various substituents at the 3-position or the 3′-position were measured. The δ values and β-lactam $\nu_{C=O}$ values are listed in Table I with the k_{obsd} values which were measured at pH 10.0 and 35 °C and geometrical means of MICs calculated from MIC values for *Escherichia coli* NIHJ JC-2 and *Klebsiella pneumoniae* SRL-1. Further chemical parameters of various 3-substituents and 3-methylene substituents of cephalosporins that were used in this work are listed in Table II.

Effect of 3-Methylene Substituents on the β-Lactam Reactivity of Cephalosporins. We confirmed that both β-lactam $\nu_{C=O}$[20] and $\Delta\delta(4-3)$ values of 3-methylene-

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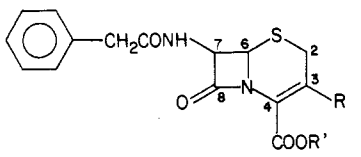
Table I. ^{13}C NMR Data, IR Kinetic Parameters, and Antibacterial Activity of Cephalosporin Having Various 3-Substituents



a, R' = Na
b, R' = $CHPh_2$

no.	R	δ[c] C-3	δ[c] C-4	δ[c] C-8	δ[c] 4-COO	β-lactam $\nu_{C=O}$, cm^{-1}	$10^2 k_{obsd}$, h^{-1}	MIC,[g] μg/mL
1a	H	118.4	132.3	166.1	169.5	1769.6	3.10	8.85
1b		122.5	126.8	164.7	160.3	1790.1		
2a	CH_3	123.1	127.4	165.1	170.7	1763.8	1.36	70.7
2b		133.6	121.6	164.8	161.0	1783.4		
3a	CH_2CN	113.9	131.8	165.6	168.5	1773.0	*e*	
3b		123.0	124.8	165.1	160.3	1793.1		
4a	$CH_2COOC_2H_5$	118.7	131.1	165.5	169.2	1768.6	1.82	>100
4b		128.3	124.2	165.0	160.7	1787.3		
5a[a]	$CH_2N^+C_5H_5$	113.0	136.1	165.2	167.7	1777.9	47.3	
6a[b]	CH_2OH	122.1	130.3	165.5	169.8			
6b		134.6[d]	121.9[d]	165.0	160.8	1791.0		
7a[a]	CH_2OCOCH_3	117.3	132.4	165.5	169.0	1771.6	11.1	25[h]
7b[a]		126.3	125.1	165.1	160.5	1791.6		
8a[a,b]	CH_2SCH_3	120.9	130.4	165.1	169.5			
9a	CH_2STet	118.9	131.8	165.4	168.5	1772.7	9.21	1.56
9b		128.6	124.7	165.1	160.6			
10b[a]	CHO	122.8	138.1	165.4	159.6			
11b	OH	166.1	103.2	166.9	164.9	1781.1		
12a	OCH_3	153.1	117.2	167.1	169.4	1765.4	*f*	17.7
12b		161.8	109.2	166.4	160.1	1780.5		
13b	$OCOCH_3$	145.1	118.5	165.4	158.8	1789.0		
14a	OSO_2CH_3	134.1	128.1	166.0	170.2	1777.4	46.5	8.84
14b		139.9	121.0	165.5	158.6	1794.0		
15a	Cl	116.6	130.0	164.8	168.2	1774.7	15.2	12.5
15b		123.8	124.0	164.5	159.4	1791.6		

[a] 7-Acylamino substituent of these compounds is (2-thienylacetyl)amino. However, the effects of the 7β-acylamino substituents on δ(C-3), δ(C-4), δ(C-8), and δ(COO) and on β-lactam reactivity are rather small. [b] ^{13}C chemical shifts of these compounds were taken from ref 17. [c] Spectral data in $CDCl_3$ solution and detailed spectral data with full signal assignment will be reported elsewhere. [d] It was regarded that the δ(C-3) and the δ(C-4) values were respectively shifted a lower and a higher field compared to that found for the acetylated compound. This is due to the effect of hydrogen bonding of $(CD_3)_2SO$ to the OH group. In the same way, the double-bond C-2 and C-1 signals in geraniol, $(CH_3)_2C{=}CH(CH_2)_2(CH_3)C(1){=}CH(2)CH_2OH$, were found to be respectively shifted to a lower (+1.3 ppm) and a higher (−3.5 ppm) field in $(CD_3)_2SO$ than in $CDCl_3$, whereas these signals were in its acetate form essentially unchanged in both solvents. An effect of hydrogen bonding of D_2O to the OH group on δ(C-3) and δ(C-4) similar to that of $(CD_3)_2SO$ may exist in Na salt. [e] Not detected because the decrease of UV absorption at 265 nm was very small. [f] Not detected because the degradation of the β-lactam ring of this compound was very slow. [g] Geometrical mean of MIC calculated from MIC values for *E. coli* NIHJ JC-2 and *K. pneumoniae* SRL-1. [h] This MIC was measured for the 7-(phenylacetyl)amino compound.

substituted cephalosporins showed linear correlations with log k_{obsd} values and that the compound having the higher β-lactam $\nu_{C=O}$ value and the larger Δδ(4–3) value showed a higher β-lactam reactivity:

$$\log k_{obsd} = 0.116\ \nu_{C=O} - 204$$

$$r = 0.9577,\ n = 5$$

$$\log k_{obsd} = 0.085\ \Delta\delta(4\text{–}3) - 0.340$$

$$r = 0.9096,\ n = 5$$

First we tried to express the ^{13}C chemical shifts of 3-methylene-substituted cephalosporins by equations similar to those used for the interpretation of the ^{13}C chemical shifts of 1-monosubstituted propenes since the substituent effect on δ(C-3) and δ(C-4) of cephalosporins is regarded as similar to that on the ^{13}C chemical shifts of β- and γ-carbon of 1-monosubstituted 2-propenes[21,22] ($H_2C{=}CHCH_2X$). We found that both ^{13}C NMR values,[24] δ(C-3)

(20) The reason why the good correlation between the β-lactam $\nu_{C=O}$ values and the log k_{obsd} values was found in this case although Boyd[8] reported a poor correlation between both the values for the 7-[(2-thienylacetyl)amino] series is probably due to the difference between the conditions of the measurement of the IR spectra of both series; the IR spectra of 7-[(phenylacetyl)amino]cephalosporinate ions and their esters were very accurately measured in dry Me_2SO and $CHCl_3$, respectively, in concentrations sufficiently diluted to take a monomeric form (see Experimental Section), whereas those of the 7-[(2-thienylacetyl)amino] series were measured as solid in KBr disks, which presumably give rather fluctuated values by strong intermolecular interactions.

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(22) It has been reported[21] that ^{13}C substituent chemical shifts of β- and γ-carbons of monosubstituted propenes (C_β-SCS and C_γ-SCS) can be exactly expressed by the $\sigma_I - \sigma_R^\circ$ dual substituent parameter equation (Taft's DSP equation).[23] But as the contribution of the σ_R° value is rather small in the analysis by Taft's equation, the relationship of β-SCS and γ-SCS with the σ_I value is shown as

$$C_\beta\text{-SCS} = 18.45\sigma_I - 2.86$$

$$r = 0.8549,\ n = 19,\ \text{excluding Cl, Br, I, Si(CH}_3)_3$$

$$C_\gamma\text{-SCS} = -7.80\sigma_I + 1.26$$

$$r = 0.7477,\ n = 19,\ \text{excluding Cl, Br, I, Si(CH}_3)_3$$

In these equations, ^{13}C-SCS is defined as a shift difference between a substituted and an unsubstituted compound.

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Table II. Chemical Parameters[a] of Various 3-Substituents and 3-Methylene Substituents of Cephalosporins

R	ι	σ_I	σ_R°
H	2.000	0.00	0.00
CH_3	2.138	−0.04	−0.11
CH_2CN	2.197	0.18	0.00
$CH_2COOC_2H_5$	2.169	0.17	
	2.230[c]	0.39[d]	0.00[c]
CH_2OH	2.215	0.05	0.00
CH_2OCOCH_3	2.216	0.14	−0.08
CH_2SCH_3		0.05	−0.10
CH_2STet[b]		0.19[d]	
CN	2.609	0.56	0.08
CHO	2.390	0.31	0.24
$COOC_2H_5$	2.369	0.30	0.18
SCH_3	2.159	0.23	−0.25
STet[b]		0.53	
		1.09	
OH	2.791	0.25	−0.40
OCH_3	2.823	0.27	−0.45
$OCOCH_3$	2.802	0.39	−0.21
$OCONH_2$		0.46[e]	
OSO_2CH_3	2.779	0.58	−0.26
Cl	2.367	0.46	−0.23

[a] These ι, σ_I, and σ_R° values were taken from ref 9, 23, 41–43. [b] STet: (1-methyl-1*H*-tetrazol-5-yl)thio. [c] The ι and σ_R° values of $CH_2NH_3^+$ were used instead. [d] The σ_I values of CH_2STet and $CH_2N^+C_5H_5$ were estimated by dividing the σ_I values for STet and $N^+C_5H_5$, respectively, by a factor of 2.8.[33] [e] The σ_I value of $OCON(CH_3)_2$ was used instead.

and δ(C-4), are linearly correlated with the σ_I values of substituents. Furthermore, the change in the $\Delta\delta$(4–3) value upon variation of the 3-methylene substituent is larger than that of the corresponding δ(C-3) and δ(C-4) values since the δ(C-3) value shows the reverse shift to that of δ(C-4) (Figure 1a). Moreover, $\Delta\delta$(4–3) values show better linear correlation with σ_I values (Figure 1b). Thus, polarization of the $C_3{=}C_4$ double bond may be an important factor determining the β-lactam reactivity of 3-methylene-substituted cephalosporins. Second, we examined the β-lactam $\nu_{C=O}$ values and found that they show a linear correlation with the σ_I values of 3-methylene substituents (Figure 1c). Thus the β-lactam reactivity of 3-methylene-substituted cephalosporins may be predicted from the σ_I values of the substituents at the 3′-position as well as the $\Delta\delta$(4–3) or β-lactam $\nu_{C=O}$ values:

$$\log k_{obsd} = 1.46\sigma_I + 0.142$$
$$r = 0.9282,\ n = 5$$

Furthermore, the δ(COO) value may also be an index for the prediction of β-lactam reactivity of this kind of cephalosporins since δ(COO) values are correlated roughly with logarithms of k_{obsd} values as well as σ_I values, although the change in δ(COO) for a variation of the 3-methylene substituent is rather small:

$$\delta(COO) = -2.73\sigma_I + 170.1$$
$$r = 0.8788,\ n = 9$$
$$\log k_{obsd} = -0.438\delta(COO) + 73.3$$
$$r = 0.8245,\ n = 5$$

Effect of the 3-Substituents on the β-Lactam Reactivity of Cephalosporins. We also studied cephalosporins with various substituents at the 3-position. β-Lactam $\nu_{C=O}$ values[20] were correlated with log k_{obsd} values (Figure 2). δ(C-3) and δ(COO) values were also correlated with log k_{obsd} values except OSO_2CH_3[26] (Figures 3a,3b). In

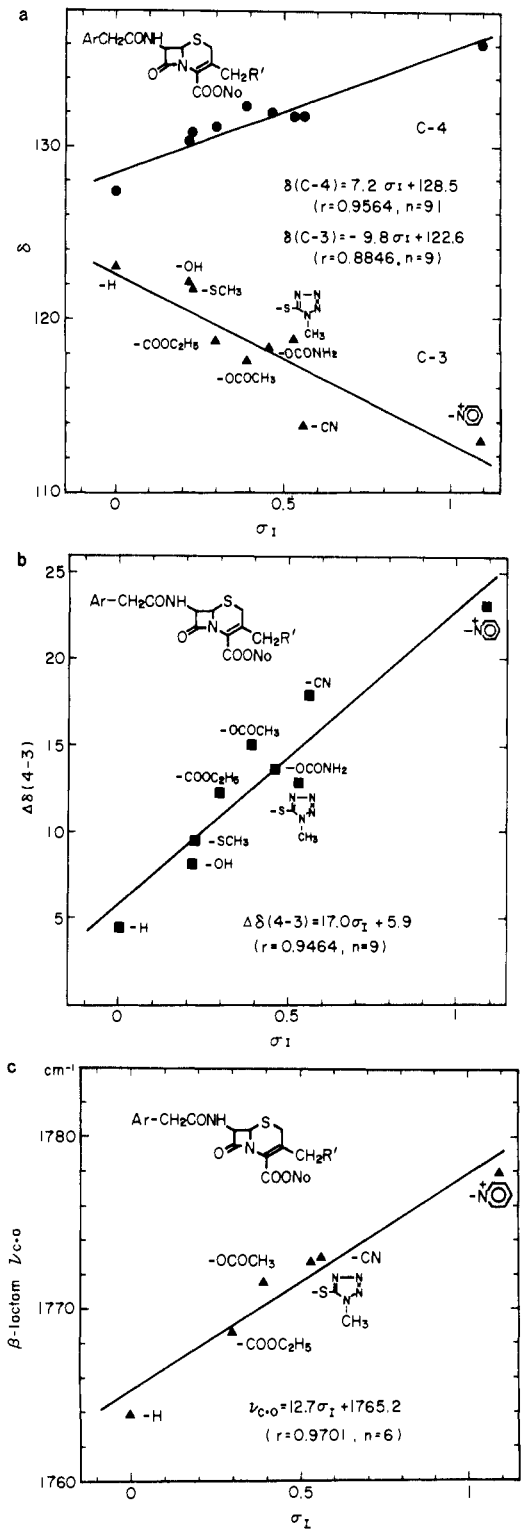

Figure 1. (a) Relationships between δ(C-3) and δ(C-4) and inductive substituent constants σ_I of various 3-methylene substituents of cephalosporins. (b) Relationship between $\Delta\delta$(4–3) values and inductive substituent constants σ_I of various 3-methylene substituents of cephalosporins. (c) Relationship between β-lactam $\nu_{C=O}$ values and inductive substituent constants σ_I of various 3-methylene substituents of cephalosporins.

(24) ^{13}C NMR data for the compound having the methoxy group at the 7α-position was used instead of those for the compound having the 3-methylene substituent $OCONH_2$ because the methoxy effects on δ(C-3) and δ(C-4) are very small.[25]

(25) Nishikawa, J.; Tori, K. *J. Antibiot.* 1981, *34*, 1645.

a similar way to that used for the investigation of 3-methylene-substituted cephalosporins, we examined the effects of the 3-substituents on ^{13}C chemical shifts and β-lactam $\nu_{C=O}$ values. However, neither ^{13}C chemical shifts nor β-lactam $\nu_{C=O}$ values could be expressed by the equation using one parameter, σ_I, alone. In regard to the substituent effect on the ^{13}C substituent chemical shifts (^{13}C-SCS) of monosubstituted ethylenes, $XHC{=}CH_2$, we have reported[21] that the ^{13}C-SCS of the C_α atom can be roughly approximated by the $\iota - \sigma_R^\circ$ DSP equation[27] and that the ^{13}C-SCS of the C_β atom can be represented as a factor of the σ_R° value. We tried to express δ(C-3) and δ(C-4) values using the same chemical parameters since both δ(C-3) and δ(C-4) were correlated to the δ(C-α) and δ(C-β) of $XCH{=}CH_2$, respectively:

$$\delta(\text{C-3}) = 1.54\delta(\text{C-}\alpha) - 70.7$$

($r = 0.9836$, $n = 8$; excluding CHO probably because of the difference of the resonance effect)

$$\delta(\text{C-4}) = 0.519\delta(\text{C-}\beta) + 64.4$$

$$r = 0.9676,\ n = 9$$

As shown below, δ(C-3) values could be expressed by a $\iota - \sigma_R^0$ DSP equation[29] (eq 1 and 4) rather than a $\sigma_I - \sigma_R^\circ$ DSP equation, while δ(C-4) values could be expressed solely by σ_R° values (eq 2 and 5, Figure 4). When the β-lactam $\nu_{C=O}$ value was analyzed by both the $\iota - \sigma_R^\circ$ equation and Taft's DSP equation, it could be represented by Taft's DSP equation (eq 3 and 6). The β-lactam $\nu_{C=O}$

(a) Cephalosporinate ions

$$\delta(\text{C-3}) = -2.5\iota - 82.9\sigma_R^\circ + 120.9 \quad (1)$$

$$r = 0.9635,\ n = 8,\ \text{excluding Cl}^{30}$$

$$\delta(\text{C-4}) = 31.3\sigma_R^\circ + 133.0 \quad (2)$$

$$r = 0.9044,\ n = 9,\ \text{excluding Cl}^{30}$$

$$\nu_{C=O} = 24.9\sigma_I + 22.7\sigma_R^\circ + 1768.7 \quad (3)$$

$$r = 0.9874,\ n = 8$$

(b) Cephalosporin esters

$$\delta(\text{C-3}) = 16.7\iota - 49.6\sigma_R^\circ + 90.5 \quad (4)$$

$$r = 0.9291,\ n = 10,\ \text{excluding Cl}^{30}$$

$$\delta(\text{C-4}) = 42.9\sigma_R^\circ + 126.5 \quad (5)$$

$$r = 0.9340,\ n = 10,\ \text{excluding Cl}^{30}$$

$$\nu_{C=O} = 22.1\sigma_I + 34.8\sigma_R^\circ + 1789.6 \quad (6)$$

$$r = 0.9773,\ n = 10$$

values were plotted against each set of δ(C-3), δ(C-4), and δ(COO) values, and two linear relationships were found among the cephalosporins, one for those with a 3-CH_2R substituent, H, or Cl and another for those with a 3-OR substituent between each pair of β-lactam $\nu_{C=O}$ values and δ(C-3) (Figure 5), δ(C-4), or δ(COO). Grouping the cephalosporins in this way was also necessary to establish linear relationships between δ values and log k_{obsd}. These exceptional effects of OR substituents are in accord with the fact that substituents having a lone pair deviate from the correlation between ι and σ_R°.[32]

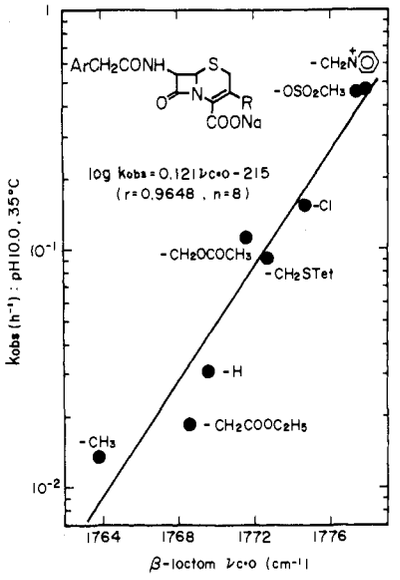

Figure 2. Relationship between β-lactam $\nu_{C=O}$ and logarithms of the alkaline hydrolysis constants k_{obsd} (log k_{obsd}) observed at pH 10.0 and 35 °C for various 3-substituted cephalosporins.

Furthermore the logarithm of the k_{obsd} value was expressed by Taft's DSP equation (eq 7). As mentioned

$$\log k_{obsd} = 2.89\sigma_I + 2.56\sigma_R^\circ - 1.42 \quad (7)$$

$$r = 0.9721,\ n = 6$$

above, we can predict the β-lactam reactivity of various 3-substituted cephalosporins using the chemical parameters σ_I and σ_R° of the 3-substituents. As for the 3-methylene-substituted cephalosporins, which are regarded as a type of 3-substituted cephalosporins, the β-lactam reactivity can be predicted more accurately by the sole σ_I value of the 3-methylene substituent because the variation of the σ_R° value corresponding to whole group at the 3-position is very small (i.e., σ_R° values of the CH_2R' groups fall in a narrow range between −0.11 and 0.00) and because the σ_I value for the 3-methylene substituent (R′) can be estimated by multiplying the value for the whole group (CH_2R') by a factor of 2.8.[33]

With respect to the effect of a 3-methylene substituent, we found[2] a linear relationship between the TSE values[34] and the Δδ(4–3) values, and therefore a parabolic relationship exists between the Δδ(4–3) values and MICs,[35] in

(26) We excluded OSO_2CH_3 from the analysis of this correlation. In order to correlate the δ values with the log k_{obsd} values for cephalosporins, we needed separate lines similar to the typical case seen in the relationships between δ values and $\nu_{C=O}$ values, which are described later in this report and include two lines: one for cephalosporins with a 3-CH_2R substituent, H, or Cl and another for those with a 3-OR substituent.

(27) ι (iota in Greek letter) is a new inductive substituent parameter that Inamoto and Masuda[28] proposed to remove a periodicity of electronegativity.

(28) Inamoto, N.; Masuda, S. *Tetrahedron Lett.* **1977**, 3287.

(29) ^{13}C-SCS of the C_α-atom in monosubstituted ethylenes has been reported[21] to exhibit three linear relationships with the ι values. However, we used the $\iota - \sigma_R^\circ$ DSP equation to simplify the analysis of δ(C-3) of cephalosporins.

(30) ^{13}C chemical shifts of the nucleus bonded to a heavy atom are known to show abnormal resonance because of spin–orbit interaction.[31] Thus we excluded Cl from the analysis of these correlation using the δ values.

(31) Morishima, I.; Endo, K.; Yonezawa, T. *J. Chem. Phys.* **1973**, *59*, 3356.

(32) Inamoto, N.; Masuda, S. *Kagaku no Ryoiki* **1979**, *33*, 105.

(33) Newman, M. S. "Steric Effects in Organic Chemistry"; Wiley: New York, 1978; p 592.

(34) The TSE values were those reported by Boyd et al.[11]

(35) MICs were those for 7-[(2-thienylacetyl)amino]cephalosporin reported by Boyd et al.[11]

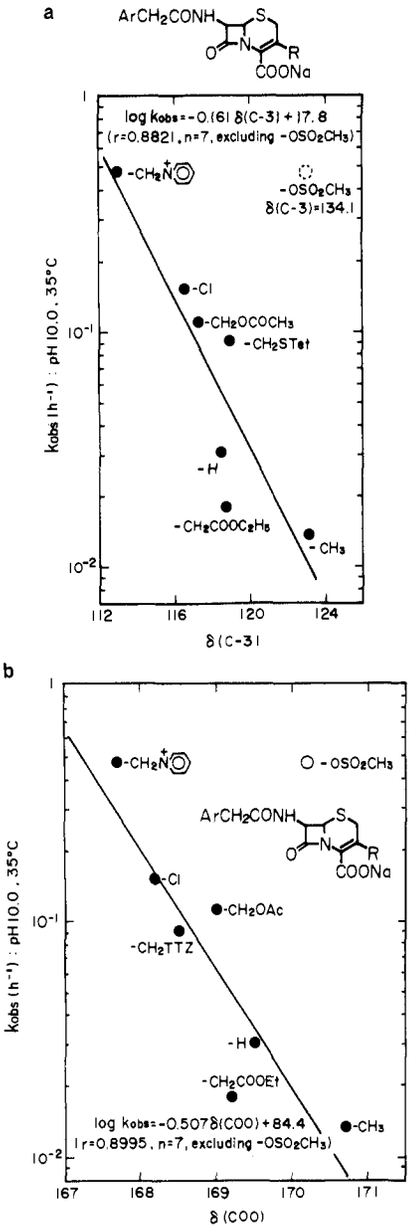

Figure 3. (a) Relationship between δ(C-3) and log k_{obsd} for various 3-substituted cephalosporins. (b) Relationship between δ(COO) and log k_{obsd} for various 3-substituted cephalosporins.

addition to the reported relationship between the TSE values and MICs:[11]

$$-\text{TSE} = 0.32\Delta\delta(4\text{–}3) + 128.7$$
$$r = 0.8556,\ n = 8$$
$$\text{MIC} = 0.22\ (\Delta\delta(4\text{–}3))^2 - 9.0\Delta\delta(4\text{–}3) + 95.2$$
$$r = 0.9116,\ n = 8$$

Further we confirmed that a linear relationship exists between the TSE values[34] and log k_{obsd} values also for 3-substituted cephalosporins including three direct 3-substituted cephalosporins with H, Cl, or OSO_2CH_3 at the 3-position as reported by Boyd:[7]

$$-\text{TSE} = 0.146 \log k_{obsd} - 20.6$$
$$r = 0.8410,\ n = 7$$

With respect to antibacterial activity,[36] we confirmed that the logarithms of k_{obsd} values[38] show parabolic cor-

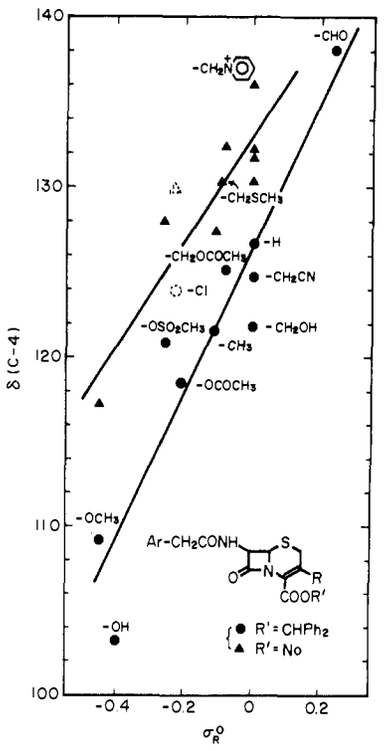

Figure 4. Relationship between δ(C-4) and σ_R° values for various 3-methylene-substituted cephalosporins.

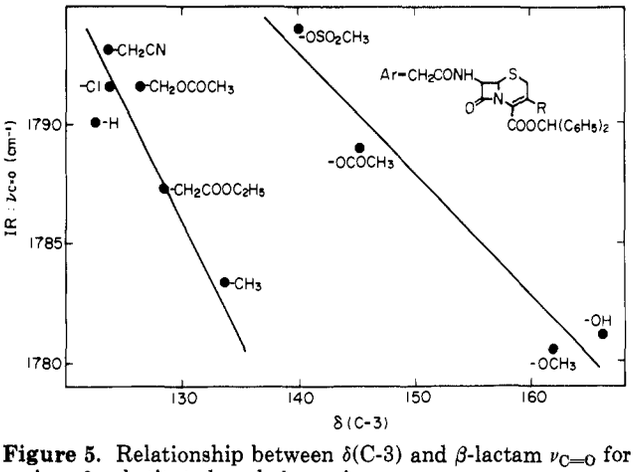

Figure 5. Relationship between δ(C-3) and β-lactam $\nu_{C=O}$ for various 3-substituted cephalosporins.

relations with MICs of 7-[(2-thienylacetyl)amino]- or 7-[(phenylacetyl)amino]cephalosporins without the classification according to the substituent at the 3-position as reported by Boyd[7] (Figure 6):

For the MICs of 7-[(phenylacetyl)amino]cephalosporins

$$\text{MIC} = 45.5\ (\log k_{obsd})^2 + 70.7 \log k_{obsd} + 33.4$$
$$r = 0.8276,\ n = 6$$

For the MICs of 7-[(2-thienylacetyl)amino]cephalosporins

$$\text{MIC} = 39.2\ (\log k_{obsd})^2 + 58.8 \log k_{obsd} + 21.9$$
$$r = 0.9248,\ n = 7$$

(36) No definite correlation between the logarithms of reciprocals of geometrical means of MICs (log $1/C$)[37] and log k_{obsd} values was found in this work.

(37) Narisada, M.; Yoshida, T.; Ohtani, M.; Ezumi, K.; Takasuka, M. *J. Med. Chem.* **1983**, *26*, 1577.

(38) We used the k_{obsd} values measured in this work for 7-[(2-thienylacetyl)amino]- or 7-[(phenylacetyl)amino]cephalosporins because the differences between the k_{obsd} values for both series are very small (ca. $\sim$0.1 s^{-1}).[5,14]

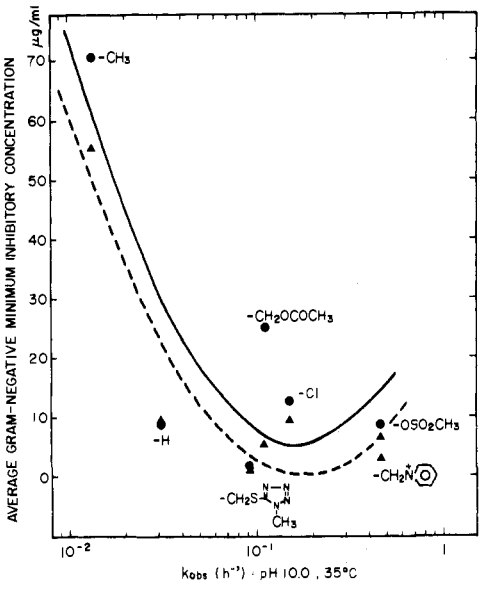

Figure 6. Relationship between log k_{obsd} and MICs for various 3-substituted cephalosporins: (▲) the plot of arithmetic average of MIC of 7-[(2-thienylacetyl)amino]cephalosporin against five Gram-negative test organisms reported by Boyd et al.[11] against log k_{obsd}, (●) the plot of geometrical mean of MIC (see Table II) of 7-[(phenylacetyl)amino]cephalosporin against log k_{obsd}.

Conclusion

We identified parameters for predicting of the β-lactam reactivity: (i) the β-lactam $\nu_{C=O}$ value is a good index for 3-substituted cephalosporins, (ii) ^{13}C NMR chemical shifts δ(C-3) and δ(COO) are rough indices for 3-substituted cephalosporins when cephalosporins are classified into the two groups of those with an OR substituent and those with a H, Cl, or CH_2R substituent, (iii) the Δδ(4-3) value is a good index for the 3-methylene-substituted cephalosporins. After analyzing these parameters, we concluded that the β-lactam reactivity of 3-substituted cephalosporins can be explained by the linear combination of inductive and resonance effects of substituents at C-3 and that of 3-methylene-substituted cephalosporins by the inductive effect of the substituents at C-3′.

The antibacterial activity of cephalosporin may be predictable from the σ_I and $\sigma_R°$ of the substituent at C-3 after prediction of the β-lactam reactivity when the parabolic relationship between the antibacterial activity and the β-lactam chemical reactivity is taken into account. We consider that a similar prediction may be applicable to cephem derivatives, when the substituents at 7α-, 7β-, and 1-positions are fixed. However, the minimal point and the curvature of the parabola may vary depending upon the nature of the substituents at C-1 and C-7 because the atom at the 1-position strongly affects the β-lactam reactivity,[37,39] the 7β-substituent may mainly affect the binding affinity to the target enzymes,[40] and the 7α-methoxy substituent sterically protects from an attack of β-lactamase and affects on another factor of the antibacterial activity of 3-cephem derivatives.[37]

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Registry No. **1a**, 92096-29-2; **1b**, 36923-19-0; **2a**, 39647-23-9; **2b**, 29126-12-3; **3a**, 92096-34-9; **3b**, 85659-59-2; **4a**, 92096-30-5; **4b**, 92096-35-0; **5a**, 92096-31-6; **6a**, 51761-82-1; **6b**, 35246-64-1; **7a**, 26382-85-4; **7b**, 41128-81-8; **8a**, 92096-38-3; **9a**, 70688-01-6; **9b**, 67366-01-2; **10b**, 35246-65-2; **11b**, 54639-48-4; **12a**, 92096-36-1; **12b**, 51761-93-4; **13b**, 51761-94-5; **14a**, 92096-32-7; **14b**, 92096-37-2; **15a**, 92096-33-8; **15b**, 63821-56-7.

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Peptide Sweeteners. 6. Structural Studies on the C-Terminal Amino Acid of L-Aspartyl Dipeptide Sweeteners

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Stereochemical and structural aspects of the variations in the C-terminal residue of L-aspartyl-L-phenylalanine methyl ester have been investigated. Novel configurational analogues such as L-aspartyl-D-alanine benzyl ester and L-aspartyl-D-α-aminobutyric acid benzyl ester were found to be sweet. In addition, chiral and achiral α,α-dialkylglycine and α-aminocycloalkanecarboxylic acids were incorporated into the dipeptides. The L-aspartic acid based dipeptide derivatives of α-aminoisobutyric acid methyl ester, α-aminocyclopropanecarboxylic acid methyl ester, α-aminocyclobutanecarboxylic acid methyl ester, and α-aminocyclopentanecarboxylic acid methyl ester are sweet. Dipeptides with α-aminocyclohexanecarboxylic acid methyl ester and α-aminocycloheptanecarboxylic acid methyl ester are bitter, whereas the analogues with α-aminocyclooctanecarboxylic acid methyl ester, α,α-diethylglycine methyl ester, and α-aminoisobutyric acid benzyl ester are tasteless. Aspects on chirality and effective volume of the C-terminal residue are discussed and correlated with taste.

Since the discovery of L-aspartyl-L-phenylalanine methyl ester,[1] structure–taste studies have shown that the aspartic acid residue is essential for the sweet taste and can be substituted only by an aminomalonate residue.[2] However,

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