Table VII. Statistical Analysis of the Effect of 5% Compound 16, 5% Acycloguanosine, and 8% Arildone Applied Five Times Daily for 4 Days on the Development of Herpetic Vesicles in Guinea Pigs Infected Intradermally with Herpesvirus Hominis, Type 1, AA Strain[a]

medication	mean vesicle score on the following days postinfection			
	day 1	day 2	day 3	day 4
5% 16	0.5	0.75	0.50	0.42
5% acycloguanosine[b]	0.58	0.83	1.16	1.0
8% arildone	0.33	0.83	0.92	0.83
placebo	0.66	1.50	1.75	1.42
nonmedicated	0.66	1.33	1.58	1.66

[a] Treatment started 24 h postinfection. [b] Commercial ointment.

was concentrated to dryness. The residual oil was distilled: yield 3.7 g (72.5%); bp 188–192 °C (0.04 mm). Anal. ($C_{21}H_{34}ClO_7P$) C, H, Cl.

Diethyl [2-(4-Acetoxyphenoxy)ethyl]phosphonate (27). A solution of 10.3 g (0.4 mol) of 2-(4-acetoxyphenoxy)-2-bromoethane and 6.6 g (0.04 mol) of triethyl phosphite was heated to 180–190 °C for 2 h and then heated to 55–60% °C for 2 days. The solvent was removed in vacuo, and the residual oil was partitioned between benzene and water. The organic layer was separated, washed, and dried. Removal of the solvent gave 30.8 g of oil, which was subjected to column chromatography on silica gel and eluted with 30% ethanol–70% ether, v/v, and 11.5 g of oil was obtained. Anal. ($C_{18}H_{28}ClO_6P$) C, H, Cl.

Diethyl [2-(4-Hydroxyphenoxy)ethyl]phosphonate (28). To a solution of 8 g (0.025 mol) of **27** in 8 mL of CH_2OH was added 80 mL of 40% $(CH_3)_2NH$. The solution was stirred for 2 h at room temperature and then evaporated in vacuo. The residual oil was partioned between 300 mL of $(C_2H_5)_2O$ and 50 mL of H_2O. The organic layer was washed and dried, and the solvent was removed, leaving a solid. The material was recrystallized from $(C_2H_5)_2O$: yield 5 g; mp 94–95 °C. Anal. ($C_{12}H_{19}O_5P$) C, H, P.

Diethyl [6-(2-Chloro-4-methoxyphenoxy)hexyl]-phosphonate (16). A solution of 10 g (0.03 mol) of 6-(2-chloro-4-methoxyphenoxy)hexyl bromide in 5.2 g (0.03 mol) of triethyl phosphite was heated to 180–190 °C, and the ethyl bromide formed was allowed to distill. After 2 h, the solution was distilled in vacuo: yield 4.6 g (39%); bp 195–197 °C (0.005 mm). Anal. ($C_{17}H_{28}ClO_5P$) C, H, Cl.

Guinea Pig Skin Infection with Herpes Virus Type 1. Albino guinea pigs, Hartly strain, weighing 350–400 g were infected with undiluted Herpesvirus hominis type 1, AA strain. An area 12 mm in diameter was marked on one epilated flank and 0.05 mL of an undiluted virus suspension was placed in the circle and injected intradermally with a Sterneedle vaccination gun. Starting 24 h postinfection, medication was applied five times daily for 4 days by gently massaging approximately 0.2 mL of the appropriate drug in a cream formulation or placebo cream into the site of infection with a fresh finger cot over rubber gloves.

Evaluation of Clinical Results. The animals were scored by a person not involved in the medication process in order to eliminate bias in evaluating the clinical effects. Guinea pigs were scored on the basis of severity of herpetic vesicles, by using 0–3 range in 0.5 increment. The scores were recorded in a notebook but not examined by the scorer until the experiment was terminated. The data was analyzed by an analysis of variance.

Lesion Sampling for Virus Content. Six guinea pigs from each group were euthanized daily for 5 consecutive days starting 24 h postinfection. The site was scraped vigorously with a sterile disposable scalpel, and recovered material was placed in a Kontes glass tissue grinder tube containing 4.0 mL of a balanced salt solution in an ice bath. The scrapings were triturated and sedimented by low-speed centrifugation. The opalescent supernatant was divided into three aliquots and stored at −70 °C for virus titer determination and protein analysis.

Virus Quantitation Assay. Monolayers of BSC-1 cells were prepared in Costar cluster dishes, each dish containing six wells of 35-mm diameter. The supernatants prepared from the skin scrapings were thawed and diluted in Eagles medium supplemented with 2% fetal calf serum from 10^{-1} to 10^{-3}. One milliliter of each dilution was added to the wells (in triplicate) after the growth medium was removed. The virus was allowed to adsorb for 1 h at 37 °C in a 5% CO_2 atmosphere, after which the residual material was removed, and 5.0 mL of a mixture of equal parts of 2X medium 199 supplemented with 5% fetal calf serum and 1% agarose (Oxoid, agar no. 1), maintained at 43 °C, was added. The agar was allowed to gel, and the dishes were then incubated at 37 °C in a 5% CO_2 atmosphere for 4 days. At the end of the incubation period, the cells were fixed to the surface of the well with 1.0 mL/well of 1% formalin containing 0.2% sodium acetate and stored at 4 °C for 24 h. The agar was gently removed from the wells, the monolayers were stained with a solution of Crystal violet in formalin, and the plaques were counted. The minimal amount of drug carried over the virus quantitation assay was shown to have no effect on plaque formation.

Registry No. **3** (X = H; Y = 4-AcO; Z = O; n = 2), 89210-89-9; **3** (X = 2-Cl; Y = 4-CH_3O; Z = O; n = 6), 56219-58-0; **6**, 73515-00-1; **7**, 73514-99-5; **8**, 73515-01-2; **9**, 73514-97-3; **10**, 73514-98-4; **11**, 73514-96-2; **12**, 73514-95-1; **13**, 89210-90-2; **14**, 89210-91-3; **15**, 89210-92-4; **16**, 73514-87-1; **17**, 73514-91-7; **18**, 73514-90-6; **19**, 73514-88-2; **20**, 89210-93-5; **21**, 73515-02-3; **22**, 73514-93-9; **23**, 89210-94-6; **24**, 89210-95-7; **25**, 89210-96-8; **26**, 73514-92-8; **27**, 89210-97-9; **28**, 89210-98-0; 1-(2-chloro-4-methoxyphenoxy)-4-iodobutane, 73523-71-4; diethyl 2-oxopropyl phosphonate, 1067-71-6; triethyl phosphonoacetate, 867-13-0; triethyl phosphite, 122-52-1.

Substituent Effects on Reactivity and Spectral Parameters of Cephalosporins[1]

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The chemical reactivity of a series of cephalosporins is examined as a function of the substituents at positions 3 and 7. In most cases, the nature of the C_7 side chain has a minor influence on the β-lactam reactivity. But in the case of amino-containing C_7 substituents, when intramolecular nucleophilic attack may occur, the reactivity may be greatly increased. The spectroscopic and structural characteristics of the β-lactam linkage do not correlate with the chemical reactivity of studied compounds. The hydrolysis rates are linked neither with the IR frequency or ^{13}C NMR chemical shift of the carbonyl β-lactam nor with the geometry of the β-lactam ring. However, a relationship is confirmed between the β-lactam ring opening rate and the polarity of the C_3–C_4 double bond, reflected in the different ^{13}C NMR chemical shifts of those atoms. The results are an experimental verification of the theoretical calculations of Boyd et al. on cephalosporin model compounds, which foresee that a C_3 substituent could favor the opening of the β-lactam cycle by stabilizing a transition state involved in alkaline hydrolysis.

The penicillins and cephalosporins are β-lactam antibiotics that inhibit the peptidoglycan transpeptidation step by inactivating certain enzymes involved in the synthesis of bacterial cell walls. Because of the structural analogy

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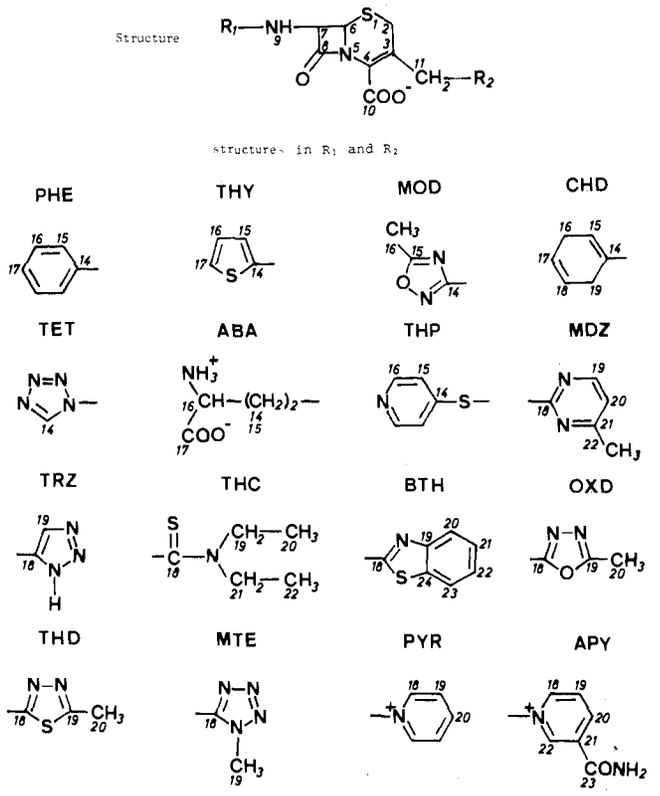
Chart I. General Structure and Structures in R_1 and R_2 for Table I



between penicillin and the C-terminal D-Ala-D-Ala of one peptidoglycan strand, Tipper and Strominger[2] have suggested that the enzyme mistakes the antibiotic as its natural substrate, the non-cross-linked peptidoglycan. Frère et al.[3] have isolated a small enzyme fragment bound to the antibiotic. This fragment contains a serine residue forming an ester bond with the penicillin. Strominger's group[4] has confirmed these observations on the enzyme of another bacterial strain. Because the acylation of the transpeptidase is necessary for antibacterial activity, the chemical reactivity of the penicillins' and cephalosporins' β-lactam should reflect their antimicrobial activity. The β-lactam function of these antibiotics is much more reactive than free amides. This is generally demonstrated by the loss of amide resonance, due to the strain in the bicyclic ring. Morin et al.[5] have correlated the IR frequency of β-lactam carbonyl and the biological activity of a series of rather different compounds. They assumed that this CO frequency reflects the acylating power. The data of Indelicato et al.[6] tend to support this assumption if one assumes that the ease of hydrolysis is directly related to the acylating power. These authors have shown that for four antibiotics the IR frequency of the β-lactam carbonyl is correlated to the base hydrolysis rate. Sweet and Dahl[7] have compared the crystal structures of two penicillins and three cephalosporins and have also considered the loss of amide resonance and a good predictor of the biological activity of the β-lactam-type antibiotics. They also proposed that antibacterial power is related to three characteristic structural features, viz., a pyramidal nitrogen atom, a short C–O bond, and a long C–N bond.[8] This hypothesis is based upon studies on the effects of modification of the β-lactam characteristics on antibacterial actions. In the cephalosporins, the β-lactam reactivity is influenced by the substituents at positions C_3 and C_7.[9] Theoretical calculations have shown that the 3-methylene substituent can influence the charge density on the β-lactam carbonyl.[10] However, ^{13}C[11-17] and ^{15}N[11] NMR studies do not reveal any

Table I. List of Cephalosporins and Related Compounds[a]

no.	substituents[b] R_1	R_2	name
1	H	H	7-ADCA
2	H	$OC^{18}OC^{19}H_3$	7-ACA
3	PHE-$C^{13}H_2C^{12}O$	H	
4	THY-CH_2CO	H	
5	MOD-CH_2CO	H	
6	PHE-OCH_2CO	H	
7	PHE-$CH(NH_2)CO$	H	cephalexin
8	CHD-$CH(NH_2)CO$	H	cephradine
9	THY-CH_2CO	OH	
10	PHE-OCH_2CO	OH	
11	THY-CH_2CO	S-CH_3	
12	THY-CH_2CO	S-MDZ	
13	MOD-CH_2CO	S-TRZ	
14	THY-CH_2CO	S-TRZ	
15	THY-CH_2CO	S-THC	
16	THY-CH_2CO	S-BTH	
17	MOD-CH_2CO	S-OXD	
18	MOD-CH_2CO	S-THD	
19	TET-CH_2CO	S-THD	cefazolin
20	THY-CH_2CO	S-THD	
21	THY-CH_2CO	S-MTE	
22	THY-$CH(C^{20}OONa)CO$	S-MTE	
23	THY-$CH(C^{20}H_3)CO$	S-MTE	
24	THY-CH(Br)CO	S-MTE	
25	ABA-CH_2CO	$OCOCH_3$	cephalosporin C
26	THY-CH_2CO	$OCOCH_3$	cephalothin
27	THP-CH_2CO	$OCOCH_3$	cephapyrin
28	PHE-CH_2CO	$OCOCH_3$	
29	PHE-OCH_2CO	$OCOCH_3$	
30	THY-CH_2NHCO	$OCOCH_3$	
31	THY-CH_2CO	PYR	cephaloridine
32	PHE-OCH_2CO	PYR	
33	THY-CH_2CO	APY	

[a] See Chart I for general formula and for formulas in R_1 and R_2. [b] Superscript numbers refer to position numbers.

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Table II. Kinetic and Spectroscopic Data

no.	$k \times 10^2$ h^{-1} [a] at pH 9.6	$k \times 10^2$ h^{-1} [a] at pH 10	UV A_{max}, [b] nm	IR $\bar{\nu}_{CO}$, [c] cm^{-1} salt	IR $\bar{\nu}_{CO}$, [c] cm^{-1} acid	δ_{C_8}, [d] ppm	$\Delta\delta$, [e] ppm
1		0.97 [f]				171.6 [g]	4.7
2	3.7		268	1801		171.9 [g]	15.6
3	1.7	3.3 [f]	261	1746		166.8 [g]	4.2
4		3.8 [f]				166.7 [h]	4.3
5	2.1		261		1776	166.8	4.1
6		4.9 [f]				166.4 [h]	4.2
7	5.8	14.5 [i]	265	1759		166.5 [h]	4.7
8	5.6	6.7 [i]	265	1760		166.5 [g]	4.5
9						167.2 [h]	8.2
10		8.4 [f]					
11		7.9 [f]				166.8 [h]	9.5
12	1.9 [j]		268		1786	167.0	9.7
13	2.6		264			167.0	9.7
14	2.8		272		1778	166.6	9.2
15	6.7		272	1766		166.8 [k]	9.5
16	9.5		282		1779	164.0 [l]	13.3
17	10.5		263		1770	167.1	12.4
18	10.4		268		1769	167.1	12.3
19	10.7	21.7 [i]	265	1755		166.5 [h]	12.2
20	12.0		274		1775	167.0 [h]	12.8
21	12.6		273		1782	167.0 [k]	12.6
22	12.2		271	1760		166.4	12.5
23 [m]	11.2		267		1776	167.0	13.1
24	21.0		269		1783	167.1	12.5
25	11.9	37.4 [f]	264	1755		167.3 [g]	12.5
26	14.7	34.9, [f] 30.0 [i]	265	1754	1776	167.1 [g]	15.1
27	15.7		261	1771		166.7 [k]	15.1
28		36.0 [f]				167.2 [n]	15.3
29		37.8 [f]				166.8 [h]	15.0
30	>300		261			168.8	15.0
31	53.0	67.5, [f] 108 [i]	256	1762		167.1 [h]	22.8
32		85.7 [f]					
33	57.0		265	1775		167.0 [k]	22.6

[a] k = pseudo-first-order rate constant of base hydrolysis. [b] A_{max} = maximum value of the UV absorption due to the 3-cephem chromophore. [c] $\bar{\nu}_{CO}$ = IR absorption of the β-lactam carbonyl. [d] δ_{C_8} = carbon-13 chemical shift of the β-lactam carbon atom. [e] $\Delta\delta$ = difference between carbon-13 chemical shifts δ_{C_4} and δ_{C_3}. [f] Data from ref 6. [g] Data from ref 13. [h] Data from ref 11. [i] Data from ref 19. [j] The hydrolysis of this compound presents two steps: an apparent stability during the first hours, followed by a degradation characterized by a pseudo-first-order rate constant ($k = 1.9 \times 10^{-2}$ h^{-1}); therefore, this constant should not be compared with the other rate constants. [k] Data from ref 14. [l] In Me_2SO-d_6. [m] Contains two isomers. [n] Data from ref 12.

significant variation of the chemical shift of these atoms by changing the substituents. The C_3 methylene substituent would then influence the β-lactam reactivity indirectly.

The objective of the present study was to examine the substituent effects on hydrolysis rate and on spectroscopic characteristics of the bicyclic fused ring system. We were mainly interested in the influence of the C_3 methylene substituent. This choice was guided by the simplicity of the model: indeed, this substituent does not correspond to a part of the transpeptidase natural substrate. Consequently, the spatial conformation of this substituent would only have a minor importance during the antibiotic–enzyme interaction. The effect of the C_3 substituent would only be indirect: it would influence the β-lactam reactivity mostly by electronic effects.

Results and Discussion

Influence of Substituents on β-Lactam Reactivity. We measured the hydrolysis rate constants of cephalosporin derivatives listed in Table I. The rate constants obtained by an UV method can be found in Table II. We also did some kinetic measurements by ^{1}H NMR. We measured the hydrolysis rate of the β-lactam and the acetate expulsion rate of **2**. The ring-opening rate was determined by following the time evolution of the peak integral corresponding to H_7. The opening of the β-lactam ring shifts this signal at 0.2 ppm to high field. The acetate expulsion rate was measured by following the decreased of the signal integral of the resonance of the acetate CH_3 protons as a function of time. The signal decrease is compensated by the appearance of the free acetate Me resonance, and the sum of these two integrals is constant.

The ring-opening rate constant is 1.7 (±0.3) × 10^{-2} h^{-1}, and the acetate expulsion rate constant is 2.4 (±0.2) × 10^{-2} h^{-1}.[18]

We have assumed that the opening of the β-lactam ring was simultaneous with the expulsion of the acetate group. Indeed, if this expulsion would occur before the ring opening, the leaving rate of the acetate in different compounds would be comparable. The substituent in the C_7 position seems to be very distant to have a significant influence on the expulsion of the acetate group if the latter precedes the opening of the β-lactam cycle. On the other hand, if the two reactions are simultaneous, the substituent in the C_7 position might influence the expulsion of the

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Table III. Carbon-13 Chemical Shifts (in Parts per Million from DSS)

C atom	5	12	13	14	16[a]	17	18	22	23[b]		24	30
2	31.1	29.3	29.5	29.2	26.6	29.2	29.4	29.1	29.0	29.0	29.3	27.9
3	125.0	122.8	122.9	122.8	118.8	121.1	121.2	120.8	120.5	120.4	121.1	118.9
4	129.1	132.5	132.6	132.0	131.3	133.5	133.5	133.3	133.6	133.5	133.6	133.9
6	59.3	60.0	60.0	60.8	57.3	59.9	60.0	60.0	60.0	60.1	59.8	60.6
7	61.5	61.5	61.7	61.8	58.6	61.8	61.7	61.5	61.8	61.6	61.4	62.6
8	166.8	167.0	167.0	166.6	164.0	167.1	167.1	166.4	167.0	166.9	167.1	168.8
10	172.8	170.9	170.5	170.0	163.5	170.5	170.6	170.3	170.2	170.3	170.1	170.9
11	21.2	35.5	40.7	40.2		38.2	40.9	38.7	39.0	39.0	39.0	66.7
12	172.8	176.1	172.6	175.6	169.8[c]	172.6	172.7	175.2[c]	180.0	180.0	172.7	161.2
13	35.1	38.7	35.2	38.4	35.7	35.1	34.9	58.9	44.0	43.9	32.1	41.2
14	167.2	138.3	167.2	137.9	136.8	167.2	167.2	139.7	146.0	145.6	138.8	145.0
15	181.6	129.9	181.6	129.6	126.2	181.6	181.5	129.4	129.7	128.0	130.0	129.6
16	14.2	129.9	14.1	129.6	126.2	14.1	14.2	129.4	129.7	128.0	130.0	129.6
17		128.1		127.9	124.3[d]			128.7	127.6	127.8	129.4	127.7
18		171.4	139.0	137.9	169.7[c]	166.6	168.8	156.0	156.1	156.1	156.2	176.5
19		159.6	134.3	134.3	152.5	170.3	173.2	36.7	36.7	36.7	36.7	22.9
20		119.9			121.1[e]	13.0	17.6	175.0[c]	21.4	20.8		
21		172.3			124.8[d]							
22		25.6			121.6[e]							
23					126.5							
24					134.7							

[a] In Me_2SO-d_6. [b] Contains two isomers. [c-e] Assignments may be interchanged.

acetate group indirectly by acting on the opening of the β-lactam cycle. The rate constant of the expulsion of the acetate group determined by ^1H NMR is 13.8 (±1.2) × 10^{-2} h^{-1} for **26** and 10.1 (±0.7) × 10^{-2} h^{-1} for **25**; these values are much larger than the one obtained for the acetate leaving rate constant of **2** (k = 2.4 × 10^{-2} h^{-1}). The presence of a RCH_2CONH substituent in C_7 increases the expulsion rate of the acetate group. This expulsion then occurs simultaneously with the opening of the β-lactam cycle.

In another series of experiments, the expulsion rate of the pyridinium group during the hydrolysis of **31** was measured by NMR. This rate was evaluated by following the decrease, as a function of time, of the peak integral corresponding to H_{18} bound to C_{18}. A rate constant of 22 (±2) × 10^{-2} h^{-1} was obtained by NMR, whereas a ring-opening rate constant of 53 (±2) × 10^{-2} h^{-1} was obtained by UV. Once again this difference could be explained by the fact that NMR and UV experimental conditions (concentration and solvent) are not quite similar.

(a) Effect of the C_7 Side Chain. This substituent generally contains an R group (often an unsaturated cycle) attached to the β-lactam ring by an acetamide linkage: $R\text{-}CH_2CONH\text{-}$.

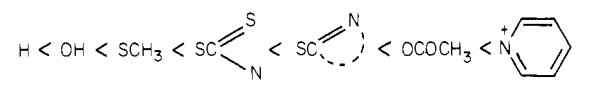
This moiety is substituted several times (**7**, **8**, and **22–24**) or lengthened (**25** and **27**) and modified once (**30**). We differentiated two types of substituents: the one with a normal or lengthened acetamide linkage (type A) and the one with a substituted or modified acetamide linkage (type B). All the A-type substituents have a similar effect on the β-lactam cycle reactivity. For the cephalosporins with the same C_3 methylene substituent, one observes rather similar reactivities almost independent of the A-type C_7 side chain.

Among the B-type substituents, several have an effect comparable to the effect of the A-type substituents (**22** and **23**), whereas the others can increase the reactivity by a factor of two (**7**, **8**, and **24**) or much more (**30**). Compound **24** presents a particular effect of substitution at the C_{13} position, but this increase of reactivity is not yet explained.

The increased hydrolysis rate of **7** and **8** was attributed to an intramolecular nucleophilic attack.[19-21] Such a mechanism could also explain the great reactivity of **30** containing an NH function in the C_7 side chain. A ^1H NMR study of this compound showed that the opening of the β-lactam ring and the expulsion of the acetate group are simultaneous. The kinetic results obtained by ^1H NMR also confirmed the very high hydrolysis rate.

For all the molecules studied here, we noticed that the B-type substituents retain or increase the β-lactam reactivity in comparison with the A-type substituents.

(b) Effect of the C_3 Side Chain. By comparing measured reactivities, we can classify R_2 substituents based on their influence on the β-lactam opening rate; we obtained the following sequence:



These substituents can affect the hydrolysis rate by inductive effect and by their more or less pronounced leavability.[22]

Influence of Substituents on Spectroscopic Characteristics. The spectroscopic analysis was performed in aqueous solution by ^{13}C NMR (Table III) and in the solid state by IR (Table II).

(a) The β-Lactam Bond. The only significant difference observed by NMR is the decrease of the C_8 chemical shift after introduction of a substituent in the NH_2 function bonded to C_7.

The β-lactam IR frequency varies significantly in this series of compounds. A difference of ~20 cm^{-1} is observed between IR frequencies of acidic forms (~1780 cm^{-1}) and those of salt forms (~1760 cm^{-1}).

This observation confirms the previous result obtained by Green et al.,[23] and our IR data are in good agreement with those of Cocker et al.[24] for **12** and **16**.

The side chains also influence the endocyclic CO IR frequency, but it is difficult to rationalize these substituent effects because the IR data examination shows that the

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Table IV. Structural Characteristics of the β-Lactam in Different Cephalosporins

no.	bond length, Å		sum of angles around N, deg
	C=O	C-N	
19[a]	1.22 (3)[b]	1.36 (3)[b]	358
21[c,d]	1.18 (2)	1.40 (2)	354
	1.21 (2)	1.38 (2)	355
26[c,e]	1.21 (2)	1.35 (2)	359
	1.15 (2)	1.43 (2)	352
27[f]	1.21 (1)	1.38 (1)	352
31[g]	1.21 (1)	1.38 (1)	351
34[h]	1.21 (1)	1.40 (1)	354

[a] Reference 27. [b] Standard deviation in parentheses. [c] Those compounds contain two independent molecules per asymmetric unit. [d] Reference 28. [e] Reference 29. [f] Reference 30. [g] Reference 7. [h] Methyl ester with R_1 = PHE-OCH_2CO and R_2 = H.[31]

R_1 effect is not independent of the nature of R_2 and vice versa. The IR results must be cautiously compared because of the polymorphic character and the differently solvated varieties in cephalosporin series.[25] Infrared spectra of six crystalline forms of cephaloridine have been discussed; two solvated forms of cephazolin are known, and their IR spectra are slightly different.[26]

The ^{13}C NMR and IR data do not allow the evaluation of the influence of the substituents at the β-lactam function for the prediction of chemical reactivity.

The available crystallographic data[7,27-31] are summarized in Table IV. The structural characteristics do not show significant variations at the β-lactam function.

(b) The C_3–C_4 Double Bond. The different techniques used in this study do not allow a specification of the substituent effects at the β-lactam. We have then searched for spectroscopic characteristics of the cephem ring influenced by the side chain. The chemical shifts of carbons 3 and 4 are strongly dependent on the R_2 substituent, and their values are practically independent of the R_1 side chain. To evaluate this double-bond polarity that depends on the nature of R_2, we have used the chemical-shift difference ($\Delta\delta = \delta_{C_4} - \delta_{C_3}$) previously proposed.[15,17]

(3) Structure–Chemical Reactivity Relationship. No significant relationship is observed between the reactivity and the geometrical and spectroscopic characteristics of the β-lactam ring. However, the chemical reactivity is certainly a factor affecting antibacterial activity. Accordingly, our observations are in contradiction with the generally accepted hypotheses of studies dealing with the structure–activity relationship of penicillins and cephalosporins.

In the cephalosporins, the β-lactam ring is nearly planar. At present, the cephalotin and the cefazolin are among the most active β-lactam-type antibiotics. Moreover, all the penicillins studied by X-ray show a pyramidal nitrogen atom; the sum of the valence angles lies between 330 and 340° (even for the less active), while in the cephalosporins this sum is always higher than 350° (even for the most active). However, it is well known that many cephalosporins are more active than penicillins. The pyramidal character of the nitrogen atom does not represent a molecular parameter that has to be considered in the interpretation of biological activities and chemical reactivities.

If the β-lactam-type antibiotics really work as transition-state analogues of the D-Ala-D-Ala residue,[32] there is no reason why the nitrogen atom must be pyramidal.

Theoretical studies[33] on this intermediate conformation indeed indicate that the amide bond is almost planar. Boyd predicted diedral angles of 167° for α attack and 160° for β attack. The crystallographic results give a value of approximately 135° for penicillins and about 160° for cephalosporins [see, for instance, **21** (very active)].[34]

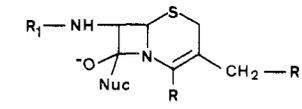
The ^{13}C NMR data (endocyclic CO chemical shifts) and IR frequencies are also in contradiction with the previous assumption.

The loss of amide resonance in the penicillins compared to the cephalosporins is not a good predictor of the reactivity.

In the following paragraphs we will examine the part played by the R_2 substituent on the β-lactam reactivity. We will distinguish three types of compounds: those without an R_1 side chain (**1** and **2**) (their reactivity is lower than the reactivity of the analogues with the same R_2 substituent), those presenting abnormally high hydrolysis rates compared to the analogues with the same R_2 substituent (**7**, **8**, **24**, and **30**), and finally the other compounds for which the hydrolysis rate is essentially dependent on the nature of R_2. In this series we will specify how R_2 influences classical cephalosporin reactivities.

This substituent strongly influences the C_3–C_4 double-bond polarity reflected by $\Delta\delta$. The effect of R_2 on the C_3–C_4 double bond was foreseen in Hermann's calculations.[10] The $\Delta\delta$ index is correlated with the logarithm of the hydrolysis rate constant for the third type compounds (Figure 1). The relationship indicates that the electron-withdrawing effect of the R_2 substituent (estimated by $\Delta\delta$) increases the β-lactam hydrolysis rate.

A generally accepted model[33] for the cephalosporin hydrolysis assumes the existence of a transition state represented by



where Nuc is a nucleophile, as the hydroxyl group in alkaline hydrolysis.

Theoretical calculations[35,36] have shown that during the α-face attack of carbon 8 by a nucleophilic agent, the C_3–C_4 double-bond polarity increases. The approach and fixation of OH^- on carbon 8 induce a negative charge increase at C_3 and a positive charge increase at C_4. Consequently, we

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(34) Note added in proof: during the preparation of this manuscript, a paper appeared on the geometrical conditions for antibacterial activities. The author compares a set of β-lactam structures, and his analysis of the three-dimensional features shows that the reduction of the geometrical requirements only to the pyramidal character of the nitrogen is not satisfactory: Cohen, N. C. *J. Med. Chem.* **1983**, *26*, 259.
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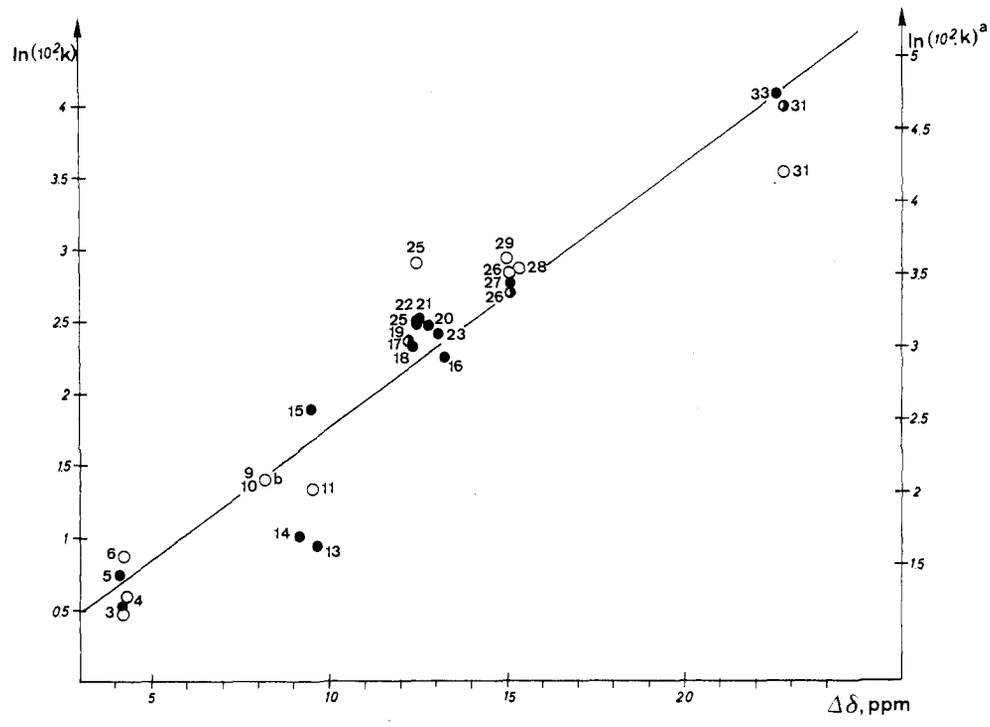

Figure 1. Relationship between hydrolysis rate constant (k) and carbon-13 chemical shift difference ($\delta_{C_4} - \delta_{C_3} = \Delta\delta$): (●) our data (left-side scale); (○) data from ref 6 (right-side scale); (◑) our data and data from ref 19 (right-side scale). Footnote *a* in figure: the scale shift of 0.71 results from the best fit between our data at pH 9.6 and the results obtained at pH 10. It corresponds to a multiplication factor of ~2 in rate constants consequent to this pH increase. This enhancement factor is in good agreement with the data of Yamana and Tsuji concerning the hydrolysis rate constants variation as a function of the pH. Footnote *b* in figure: by assuming that $\Delta\delta$ of compound **10** is 8.2 ppm and k of compound **9** is 8.4×10^{-2} h^{-1}.

Table V. Transition State Energies of Model Compounds for Different 3-Methylene Substituents and Hydrolysis Rate Constants (Average) of Corresponding Cephalosporins

R_2	TSE,[a] kcal mol^{-1}	$k \times 10^2$ h^{-1} [b] this study	$k \times 10^2$ h^{-1} [b] other results[c]
H	129.6	1.9 (3,5)	4.0 (3,4,6)
OH	131.0		8.4 (10)
SCH_3	132.2		7.9 (11)
$OCOCH_3$	133.1	14.1 (25,26,27)	35.2 (25,26,28,29)
S-THD	133.7	11.0 (18,19,20)	21.7 (19)
S-MTE	135.2	12.3 (21,22,23)	
PYR	136.6	53 (31)	87 (31,32)

[a] Values calculated by Boyd (personal communication and ref 37). [b] Numbers in parentheses are compounds numbers; because of the minor influence of the R_1 side chain on the observed rate constant, we averaged the data to obtain a general measure of k as a function of R_2. [c] From the data of ref 6 and 19.

can assume that one effect of an electron-withdrawing substituent R_2 is to facilitate the approach and the fixation of an OH^- on the β-lactam carbon.

Recent calculations on the theoretical index of reactivity of cephalosporin model compounds by Boyd et al.[37] show that this index, called transition-state energy (TSE), is strongly dependent on the R_2 side chain. It is expressed as the decrease in CNDO/2 total energy of a complex formed by placing an OH^- 1.5 Å from the α face of C_8 of a given 3-substituted 7-amino-3-cephem compared to OH^- and this cephem structure at infinite separation. If the proposed mechanism for the alkaline hydrolysis of the cephalosporins is correct and especially if the transition intermediate exists during the reaction, the theoretical index of reactivity calculated by Boyd for model compounds with different R_2 side chains must be correlated to the chemical reactivity of cephalosporins differently substituted at C_3.

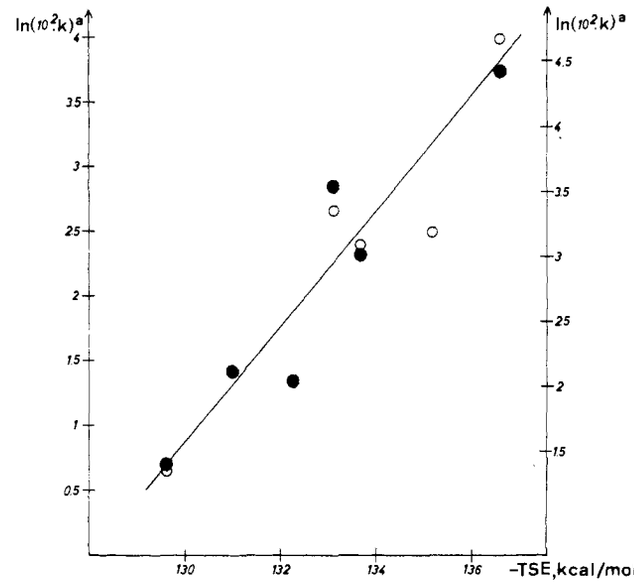

Figure 2. Relationship between average rate constants and calculated TSE: (○) our data (left-side scale); (●) average values from ref 6 and 19 (right-side scale). Footnote *a* in figure: see footnote *a* of figure 1.

The kinetic results we have obtained, those already published,[6,19] and the calculated TSE of cephalosporin model compounds can be found in Table V. The kinetic measurements are an experimental verification of the existence of this transition state used for the calculation of the theoretical index of reactivity. A good correlation, illustrated in Figure 2, indeed exists between the alkaline hydrolysis rate constants (a measure of chemical reactivity) and the transition-state energy (taken as a theoretical index of reactivity).

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The $\Delta\delta$ values also depend on the nature of R_2: it is then quite logical to find a correlation between $\Delta\delta$ and TSE as recently published by Nishikawa and Tori[15] in their work on the 3-methylene substituent effect in cephalosporins.

In this study we have shown that the β-lactam reactivity of the cephalosporins is not related to the geometrical characteristics of the cephem ring, as determined by X-ray diffraction, and that the R_1 and R_2 side chains do not modify the β-lactam spectroscopic characteristics in such a way that these modifications would allow the prediction of the hydrolysis rates.

We have also shown that the β-lactam reactivity depends upon (1) the nature of the R_1 side chain [particularly when the C_{13} is substituted by an amino group or when the methylene at position 13 is replaced by an NH function; such derivatives may undergo intramolecular amino attack at the β-lactam, and the observed opening rate is accelerated] and (2) the R_2 substituent [the more its electron-withdrawing character is pronounced, the more it stabilizes a transition intermediate, invoked during base hydrolysis of the cephalosporins]. This last observation is an experimental verification of the proposed mechanism for the cephalosporin hydrolysis. It also clearly shows that chemical reactivity is a factor affecting antibacterial activity. Our results confirm the Boyd et al.[37] correlation between the calculated chemical reactivity and the biological activity. Low chemical reactivities correspond to poor antibacterial activities, and the active cephalosporins always have relatively high hydrolysis rates.

Experimental Section

β-Lactam Compounds. Several cephalosporin derivatives (**7**, **8**, **19**, **26**, **27**, and **31**) are commercially available. Some derivatives were donated by different companies: compounds **1**, **3**, **5**, **13**, **14**, **17**, **18**, **20–24**, and **30**, Smith Kline-RIT, Genval; compounds **19** and **26**, Eli Lilly and Co., Indianapolis; compounds **2** and **25**, Glaxo Laboratories Ltd, Middelsex. Compounds **12**, **16**, **25**, and **33** were synthesized following literature procedures.[24,38,39]

UV Spectroscopy. The alkaline hydrolysis of the cephalosporins was followed by measuring the decrease of the absorption band near 260 nm (due to O=CNC=C linkage)[40] as a function of time. The kinetic measurements were performed with a UV Beckman (Model 24) spectrophotometer on aqueous solutions of cephalosporins at a concentration of 6×10^{-4} M and at 35.0 ± 0.1 °C. The reaction solution pH was stabilized at 9.60 ± 0.02 by a borate buffer solution (Merck). The ionic strength was adjusted to 0.1 by the addition of KCl. The hydrolysis rate constants were calculated by the following equation.[19]

$$\ln (A_t - A_\infty) = \ln (A_0 - A_\infty) - kt$$

where A_t, A_∞ and A_0 are the absorbances at time t, infinity, and zero, respectively. Standard deviations on rate constants are less than 10%.

NMR Spectroscopy. This was used for the following three types of studies: (1) the determination of the ^{13}C chemical shifts ($\delta_{^{13}C}$) of all the carbon atoms of the different compounds (data for compounds not yet studied by NMR are listed in Table III); (2) the kinetic measurements (^1H); (3) the mechanism of degradation (^1H and ^{13}C).

The ^1H NMR spectra were measured on a Varian XL 100/15 in the CW mode for most compounds or in the FT mode for the kinetic measurements. The hydrolysis rate constants were obtained by following the evolution of one or several peaks as a function of time. The concentration of the cephalosporins in D_2O was $2–3 \times 10^{-2}$ M; the solutions were maintained at 35 °C and stabilized at pH 9.6 by a phosphate buffer solution. Ionic strength was adjusted to 0.1 by the addition of KCl. Typical FT conditions were as follows: spectral width, 1000 Hz; flipping angle, 35°; acquisition time, 4 s; sample tube diameter, 5 mm; number of scans, 200–300.

The ^{13}C NMR spectra were run in the FT mode on two different spectrometers under the following conditions:

spectrometer	Varian XL 100/15	Bruker WM 250
operating frequency, MHz	25.2	62.9
flipping angle, deg	17-22	25
acquisition time, s	0.8	1.08
tube diameter, mm	12	10

The D_2O solutions (concentration 0.2–0.5 M) were stabilized at pH 9.6 (phosphate buffer). DSS was used as internal reference. The resonance assignments were based on spectral characteristics previously described.[13]

IR Spectroscopy. The infrared spectra of Nujol mull samples were recorded on a Beckman IR 12 spectrometer.

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Registry No. 1, 22252-43-3; **2**, 957-68-6; **3**, 27255-72-7; **4**, 34691-02-6; **5**, 88635-55-6; **6**, 10209-11-7; **7**, 15686-71-2; **8**, 38821-53-3; **9**, 5935-65-9; **10**, 10360-10-8; **11**, 26722-85-0; **12**, 88635-56-7; **13**, 88635-57-8; **14**, 88635-58-9; **15**, 88635-59-0; **16**, 3933-02-6; **17**, 88635-60-3; **18**, 88635-61-4; **19**, 25953-19-9; **20**, 26970-95-6; **21**, 32924-66-6; **22**, 88635-62-5; **23**, 88635-63-6; **24**, 88635-64-7; **25**, 61-24-5; **26**, 153-61-7; **27**, 21593-23-7; **28**, 859-07-4; **29**, 10390-44-0; **30**, 51647-23-5; **31**, 50-59-9; **32**, 18646-67-8; **33**, 3534-46-1.

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