# Hydrolysis of 3-Chloro-3-cephems. Intramolecular Nucleophilic Attack in Cefaclor

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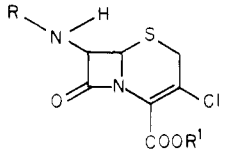
The chemical reactivity of 3-chloro-3-cephems was found to be similar to that of the correspondingly substituted 7-aminocephalosporanic acids and 12–13 times greater than that of the correspondingly substituted 7-aminodeacetoxycephalosporanic acids. Cefaclor, 7-(D-2-amino-2-phenylacetamido)-3-chloro-3-cephem-4-carboxylic acid, was found to undergo intramolecular nucleophilic attack at the β-lactam. Loss of chlorine from 3-chloro-3-cephem may be a general reaction subsequent to β-lactam opening.

Cephalosporins are β-lactam-containing antibiotics which interfere with the three-dimensional cross-linking of peptidoglycan strands by transpeptidase during the final stage of bacterial cell wall biosynthesis.[1] Because acylation of transpeptidase is necessary for antibacterial activity, the chemical reactivity of the β-lactam moiety may reflect its antibiotic activity. Substitution at the 3-methylene position has a greater effect upon β-lactam reactivity than substitution in either the 7-acylamido side chain[2,3] or the 7α position.[4] Chauvette and Pennington have recently reported the synthesis of a new class of cephalosporins in which an electronegative chloro substituent is directly attached at C-3.[5,6] Cefaclor [**1b**, 7-(D-2-amino-2-phenylacetamido)-3-chloro-3-cephem-4-carboxylic acid] is orally absorbed in dogs[7] and man[8] and more active microbiologically[9] than cephalexin [7-(D-2-amino-2-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid; cephalexin monohydrate, KEFLEX, Lilly]. Our objectives in this study were (1) to measure the substituent effect of the C-3 chlorine atom upon the β-lactam reactivity and (2) to determine if competitive intramolecular nucleophilic attack by the α-aminophenylacetyl side chain of cefaclor is involved in β-lactam hydrolysis.



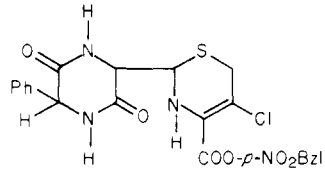
1a, R = 2-thienylacetamido; $R^1$ = H
b, R = PhCH($NH_2$)C(=O) (D); $R^1$ = H
c, R = $PhCH_2C(=O)$; $R^1$ = H
d, R = $PhOCH_2C(=O)$; $R^1$ = H
e, R = H; $R^1$ = $CH_2C_6H_4$·4-$NO_2$
f, R = PhCH($NH_2$)C(=O) (D); $R^1$ = $CH_2C_6H_4$·4-$NO_2$

## Results and Discussion

As shown in Figure 1, the β-lactam reactivity of 7-phenylacetamido-, 7-(2-thienylacetamido)-, and 7-phenoxyacetamido-3-chloro-3-cephem-4-carboxylic acids (**1c**, **1a**, and **1d**) is only 30–80% greater than the reactivity of the analogous 7-aminocephalosporanic acids, while it is 12–13 times greater than that of the analogous 7-aminodeacetoxycephalosporanic acids. These differences in chemical reactivity are reflected in microbiological activity. For example, the rates of β-lactam ring opening of the 7-(thiophene-2-acetamido)cephalosporins in Table I are inversely proportional to the minimum inhibitory concentration of these antibiotics against a variety of gram-negative bacteria. Both we[2] and Bundgaard[10] have also found that cephalosporin β-lactam reactivity correlates with the $\sigma_I$ values of 3-methylene substituents. Because the 3-chloro substituent is not only more electronegative ($\sigma_I$ = 0.47) but also one carbon atom nearer to the reaction center than is the 3-acetoxymethyl moiety ($\sigma_I$ = 0.39), one might expect that the β-lactam ring in 3-chloro-3-cephems would be considerably more reactive than in the 7-aminocephalosporanic acid (7-ACA) analogues. However, if one considers that the 3′-acetate moiety may leave in a concerted manner with the opening of the β-lactam ring[11] (a case where effects other than purely inductive may operate) and that the 3-chloro substituent may not leave by a concerted mechanism (a case where inductive effects predominate), then the similarity between both the chemical and the microbiological reactivity of the 3-chloro-3-cephems and the 7-ACA derivatives is plausible.

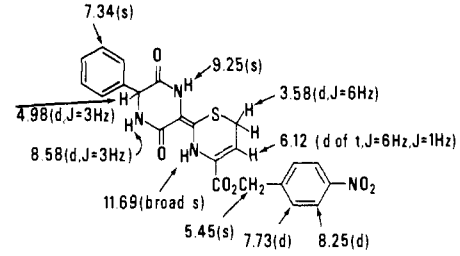
The increase in the pseudo-first-order rate of β-lactam ring opening of cefaclor (**1b**, Figure 1) over **1a**, **1c**, and **1d** is not unusual. We,[4] Bundgaard,[12] and Kanayama[13] have demonstrated that the apparent increase in reactivity of cephalexin and cephaloglycin [7-(D-2-amino-2-phenylacetamido)-3-acetoxymethyl-3-cephem-4-carboxylic acid; KAFOCIN, Lilly] (but not ampicillin) over compounds not containing α-aminophenylacetyl side chains is due to intramolecular nucleophilic attack by the α-amino moiety on the β-lactam. Attempts to isolate products from basic aqueous degradation of cefaclor were unsuccessful. However, a piperazine-2,5-dione was isolated from the acidic aqueous degradation of cefaclor[14] and a piperazine-2,5-dione was obtained by heating the *p*-nitrobenzyl (*p*-$NO_2$Bzl) ester of cefaclor **1f** in benzene under reflux.

The expected structure for the piperazine-2,5-dione was **2**, analogous to the product obtained from refluxing *p*-

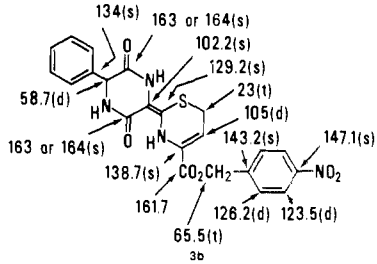


**2**

nitrobenzyl 7-[D(−)-α-aminophenylacetamido]-3-methyl-3-cephem-4-carboxylate in benzene.[15] However, elemental analysis of the product indicated the absence of chlorine and a field desorption $M^+$ of 466 suggested that the

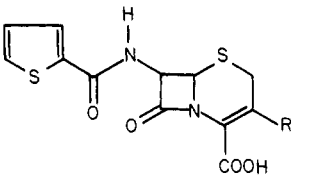



3a




3b

Table I. Gradient Plate Assay, Minimum Inhibitory Concentration[5] (MIC) Expressed in μg/mL



| R | $k_{obsd}$ × $10^5$ $s^{-1}$ [b] | *K. pneumoniae* X26 | *E. aerogenes* X68 | *Sal. heidelberg* X514 | *Shig. sp.* N9 | *E. coli* N10 |
|---|---|---|---|---|---|---|
| -Cl (1a) | 13.1 | 0.8 | 0.9 | 0.9 | 17.5 | 21.2 |
| $-CH_2OC(=O)CH_3$ (cephalothin) | 9.7[a] | 1.0 | 3.0 | 1.0 | 14 | 15 |
| $-CH_3$ | 1.07[a] | 10 | 30 | 29 | 120 | 116 |

[a] Reference 2. [b] β-Lactam ring opening at pH 10, 35 °C.

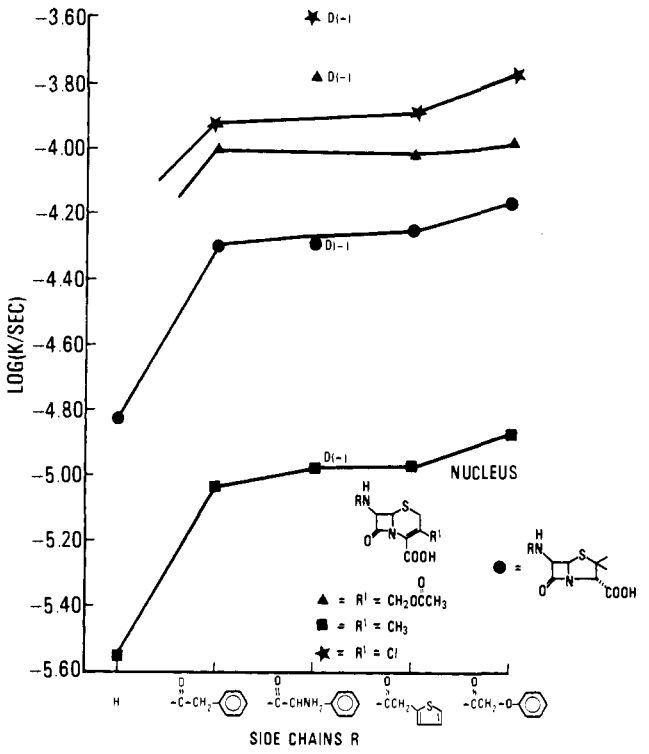



**Figure 1.** Substituent effect of acylamido side-chain modification upon observed pseudo-first-order rates of penicillin and cephalosporin β-lactam ring opening at pH 10, 35 °C. Data for ▲, ■, and ● from ref 2.

product had lost HCl. Structure **3** was assigned from the NMR spectra. The peak assignments for the proton and $^{13}C$ NMR spectra ($Me_2SO$-$d_6$) are indicated in **3a** and **3b**, respectively. Proton double-resonance experiments were in complete agreement with the proposed structure.

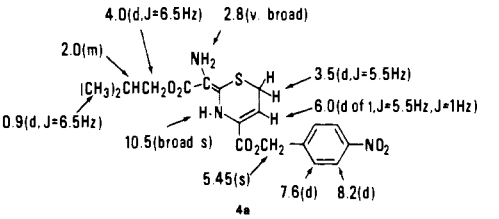
Loss of HCl from the 3-chloro-3-cephems may be a general reaction subsequent to β-lactam opening. For example, when an external nucleophile such as isobutyl alcohol reacts with **1e**, the crystalline product once again contains no chlorine and its field desorption $M^+$ of 407 suggests the loss of HCl as well as addition of isobutyl alcohol. The structure of this product **4** was also assigned from the NMR spectra. The peak assignments for the proton ($CDCl_3$) and $^{13}C$ ($Me_2SO$-$d_6$) NMR spectra are indicated in **4a** and **4b**, respectively. Proton double-resonance experiments were again in complete agreement with the proposed structure.

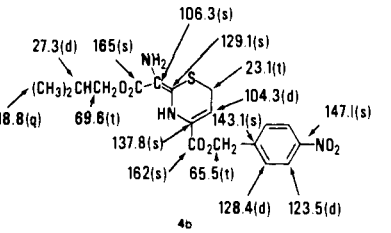
We propose the following scheme to account for a nonconcerted loss of chlorine subsequent to opening of the β-lactam, either intramolecularly by an α-amino moiety or by an external nucleophile. Structures analogous to **5**

Table II

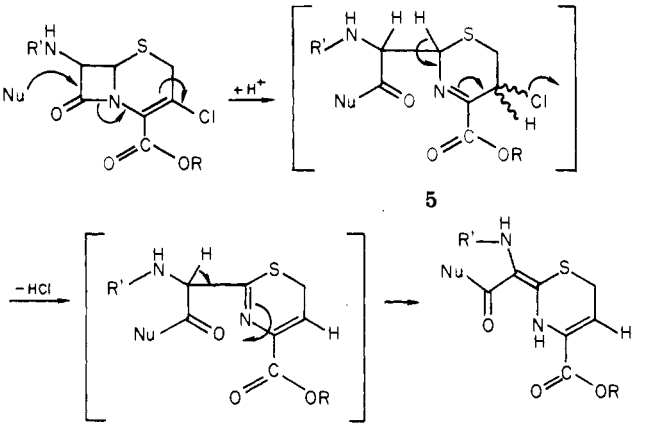
| Compd | $k_{obsd}$ × $10^5$ $s^{-1}$ | Compd ref |
|---|---|---|
| 1a | 13.1 ± 1.6 | 5 |
| 1b | 28.7 ± 2.7 | 5 |
| 1c | 12.2 ± 0.3 | *a* |
| 1d | 18.3 ± 1.1 | *a* |

[a] Belgium Patent 811 494.




(methyl substituted for chlorine) were isolated from aqueous sodium carbonate solutions of cephalexin[14] and




cephradine.[16] In these cases the C-3 methyls are nonleaving functions.

## Experimental Section

**β-Lactam Compounds.** The cephalosporins used in this study were synthesized at the Lilly Research Laboratories. Synthetic procedures for all these compounds are referenced in Table II.

**Kinetic Methods.** The cephalosporin hydrolysis rates were measured by a UV method described earlier.[12] The pseudo-first-order rates of β-lactam hydrolysis at pH 10.0, 35 °C, are listed in Table II.

**Decomposition of p-Nitrobenzyl 7-[D-(−)-α-Aminophenylacetamido]-3-chloro-3-cephem-4-carboxylate (1f).**[6] A $C_6H_6$ solution (3 L) of **1f** (2.6 g, 5.1 mmol) was stirred under reflux for 100 h. The $C_6H_6$ solution was allowed to cool and the $C_6H_6$ removed in vacuo. The mixture was chromatographed over silica gel for dry-column chromatography. Amorphous piperazine-2,5-dione **3** was eluted with 1:1 ethyl acetate–cyclohexane: 0.6 g (38%); mp 176 °C dec; field desorption $M^+$ at 466; $\lambda_{max}$ 265, 371 nm (ε 17 900, 16 600); IR (KBr) 1721 (ester), 1663 (C=C), 1640 (amide), 1340, 1510 $cm^{-1}$ ($-NO_2$); $[\alpha]^{25}_D$ +139.7° ($Me_2SO$); proton double-resonance data for **3a**, 6.12 ppm (d of t) [collapse of 3.58 ppm, (d) to a s, sharpening of 11.69 ppm (br s)], 3.58 ppm (d) [collapse of 6.12 ppm (d of t) to br s], 8.85 ppm (d) [collapse of 4.98 ppm (d) to s]. Protons at 9.25 (s), 8.58 (d), and 11.69 ppm (br s) exchanged upon $D_2O$ wash; an unsatisfactory analysis was obtained for C, H, and N, but **3** contained no Cl.

**Reaction of p-Nitrobenzyl 7-Amino-3-chloro-3-cephem-4-carboxylate (1e)**[6] **with Isobutyl Alcohol.** Chauvette and Pennington prepared **1e** by the $PCl_5$ treatment of *p*-nitrobenzyl 7-(thiophene-2-acetamido)-3-chloro-3-cephem-4-carboxylate, followed by cleavage of the imino chloride with isobutyl alcohol, which precipitates the crystalline HCl salt of **1e**.[6] If the filtrate from this procedure is allowed to stand, **4** crystallizes as the hydrochloride in yields up to 25%.

Dissolution of the HCl salt **4** in pyridine followed by precipitation with $H_2O$ gave **4** as yellow-orange crystals. Recrystallization from ethanol gave **4**: mp 114 °C dec; field desorption $M^+$ 407; $\lambda_{max}$ 263, 365 nm (ε 11 000, 8000); IR (KBr) 1721 (ester), 1669 (C=C), 1350, 1521 $cm^{-1}$ ($-NO_2$); proton double-resonance data for **4a**, 6.0 ppm (d of t) [collapse of 3.5 ppm (d) to s, sharpening of 10.5 ppm (br s)], 3.5 ppm (d) [collapse of 6.0 ppm (d) to s], 2.0 ppm (m) [collapse of 0.9 ppm (d) to s, collapse of 4.0 ppm (d) to s]. Protons at 10.5 (br s) and 2.8 ppm (v br) exchange upon $D_2O$ wash. Anal. Calcd for $C_{16}H_{21}N_3O_6S$: C, 53.07; H, 5.16; N, 10.32; S, 7.86. Found: C, 52.75; H, 4.94; N, 10.16; S, 7.56.

**Acknowledgment.** The authors thank R. R. Chauvette for samples of cephalosporins, Dr. D. Dorman for suggestions in interpreting the $^{13}C$ NMR spectra, and Dr. L. D. Hatfield for bringing to our attention that **1e** decomposes in isobutyl alcohol.

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# Cephalosporin Degradations[1]

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The acidic aqueous degradation of the 7α-aminophenylglycinamido-containing cephalosporin cephalexin (**1a**) has been examined. Two major degradation products have been isolated and characterized: 3-formyl-3,6-dihydro-6-phenyl-2,5(1*H*,4*H*)-pyrazinedione (**5**) and 3-hydroxy-4-methyl-2(5*H*)-thiophenone (**6**). By carrying out the reaction in $^{18}O$-enriched $H_2O$, the intramolecular nature of the cephalexin degradation has been demonstrated.

The chemical reactivity of β-lactam-containing antibiotics is linked to antimicrobial activity and bacterial resistance.[2] This has evoked considerable interest in the chemical degradation of cephalosporin antibiotics.[3,4] Three reports have recently appeared which detail the alkaline hydrolysis of the clinically useful antibiotics, cephalexin [7-(D-2-amino-2-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid; cephalexin monohydrate, KEFLEX, Lilly] (**1a**) and cephradine (**2**). In 1973, Cohen[5] reported that the degradation of **2** in $Na_2CO_3$ at 5 °C affords the diketopiperazine **3a**; in 1974, Yamana[6] speculated that diketopiperazine **4** forms from the hydrolysis of cephalexin at pH 8, and in 1976, Bundgaard[7] actually isolated such a compound from the alkaline hydrolysis of cephalexin.[8]

Since cephalexin possesses oral antibiotic activity, an acidic rather than a basic degradation study should better mimic any chemical reaction that might occur in the stomach. Hence, we wish to report the identification of two major products from the acidic degradation of **1a** and to propose a route to their formation. We also report herein preliminary toxicological data on the cephalexin degradation products.

## Experimental Section

**General Procedures.** Melting points were determined with a Mel-Temp apparatus and are uncorrected. Infrared spectra were determined on a Beckman IR-12 spectrometer, NMR spectra were recorded on a Varian T-60 spectrometer, and mass spectra were recorded on a Hitachi RMU-6D spectrometer at 70 eV. Elemental analyses obtained are within ±0.3% of the theoretical values.

**Cephalexin Degradation.** A solution of 1.0 g of cephalexin in 100 mL of deionized water (resulting pH 3.3) was warmed to 75 °C. Periodic examination of the solution by TLC [5:2:1:1, EtOAc–$CH_3COCH_3$–HOAc–$H_2O$; $R_f$ (cephalexin) = 0.14] revealed that most of the starting material had degraded within 90 min and two major degradation products ($R_f$ = 0.78, 0.91) were formed. The aqueous solution was then cooled and extracted with $CHCl_3$. The less polar product ($R_f$ = 0.91) was isolated from the $CHCl_3$ extract (200 mg), purified via sublimation (100 °C, 100 μ), and identified on the basis of its spectral data as the known[9] **3-hydroxy-4-methyl-2(5*H*)-thiophenone (6)**: IR (KBr) 3400–3200 (OH), 1690 (C=O), 1640 $cm^{-1}$ (C=C); $^1H$ NMR ($CDCl_3$) δ 2.1 (3