Isolation and Structure Elucidation of a Novel Product of the Acidic Degradation of Cefaclor

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Abstract □ The acidic aqueous degradation of cefaclor, an orally administered cephalosporin antibiotic, has been investigated. The most prominent peak in the high-performance liquid chromatography profile of a degraded solution of cefaclor was isolated by preparative high-performance liquid chromatography. Mechanistically, the formation of this degradent from cefaclor involves a condensation of two cefaclor degradation products in which both products have undergone contraction from a six-membered cephem ring to a five-membered thiazole ring, presumably via a common episulfonium ion intermediate.

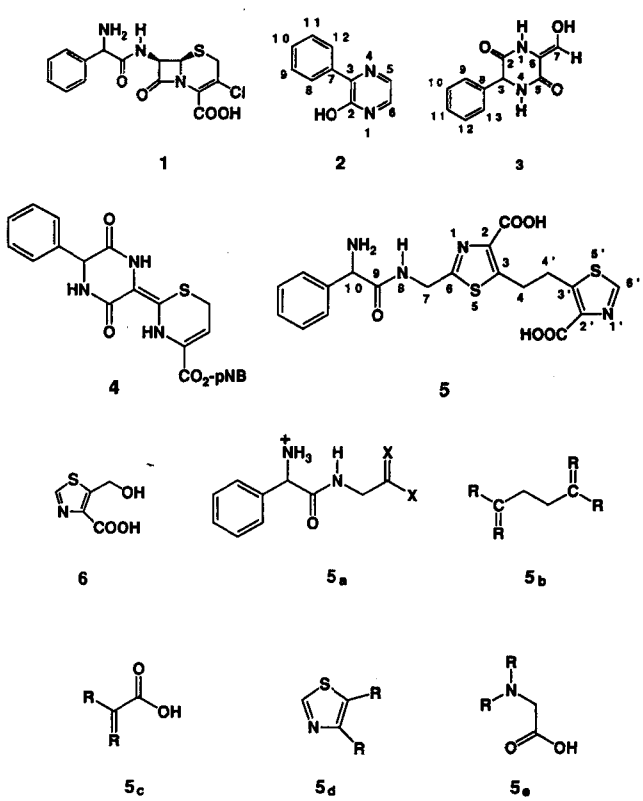
Cefaclor (1) is an orally administered cephalosporin antibiotic with a broad spectrum of antibacterial activity.[1] The antibacterial activity is dependent on the presence of the β-lactam functionality that is readily hydrolyzed under aqueous conditions.[2] This hydrolytic instability leads to chemical degradation and the formation of many products.

Previous investigations of cefaclor degradation led to the identification of only two compounds, 2 and 3.[3,4] Attempts to isolate and characterize additional degradation compounds from the numerous compounds present in the degradation solutions were not successful. A nonaqueous accelerated degradation of the *p*-nitrobenzyl ester of cefaclor by Indelicato et al.[5] resulted only in the identification of 4. In another study, Nakashima et al.[6] investigated the kinetics of the aqueous degradation of cefaclor. They concluded that cefaclor degrades via intramolecular nucleophilic attack of the primary amine of the side chain on the β-lactam moiety at neutral pH. At higher concentrations (>10 mM) it was determined that the primary amine attacked the β-lactam intermolecularly to form a dimer. However, no degradation products were isolated in the study. In studies of the metabolism of cefaclor, no metabolites have been identified, presumably because of rapid elimination and degradation in vivo.[7,8]

In this report, we present the results of our study of the degradation of cefaclor in 0.1 N HCl at room temperature. The isolation and structure elucidation of the most prominent degradation product is described. Characterization by UV, IR, NMR, and Mass (MS) spectroscopy revealed an unusual structure (5) containing two substituted thiazole rings. The formation of this compound implicates some fascinating chemistry involved in the aqueous degradation pathways of cefaclor.

Experimental Section

Cefaclor (5 mg/mL) was allowed to degrade in 0.1 N HCl at room temperature for 2 months prior to isolation. At this point, cefaclor was >90% degraded as determined by analytical reversed-phase high-performance liquid chromatography (RP-HPLC). The RP-HPLC system consisted of a Varian Vista 5500 LC pump (Walnut Creek, CA) connected to a Waters 990 photodiode array detector (Milford, MA). The HPLC was run in a gradient mode from 0 to 100% solvent B in 30 min and held at 100% B for 10 min. Solvent A was an aqueous solution containing NaH_2PO_4 (2.4 g/L) and concentrated H_3PO_4 (1 mL/L). Solvent B was a mixture of 60% acetonitrile and solvent A. The flow rate was 1.0 mL/min. A C18 column was used (4.6 × 250 mm, 5-μm particle size, YMC, Morris Plains, NJ). The UV spectra were acquired with the photodiode array detector from 200 to 400 nm with a resolution of 4 nm between diodes.



Isolation—Compounds 5 and 2 were isolated and purified by preparative RP-HPLC with gradient and isocratic solvent programs. For the initial purification, the HPLC was run in a gradient mode from 0 to 100% solvent B in 35 min and held at 100% B for 7 min (20 × 250 mm YMC C18 column, 5-μm particle size). Solvent A consisted of an aqueous solution of 0.1% acetic acid, and solvent B consisted of a mixture of 60% acetonitrile and 40% solvent A (v/v). The flow rate was 10 mL/min. Further purification was achieved by isocratic RP-HPLC with a YMC-Basic column (20 × 250 mm YMC-Basic, 5-μm particle size). In all cases, collected fractions were immediately cooled to 0 °C by placing them on ice. The solvents were removed by lyophilization to yield purified compound.

Compound 2—The synthesis of 2 was accomplished by a hybrid procedure based on the methods of Jones[9] and Lebelle[10] as follows: Phenylglycine amide was prepared by slurrying phenylglycine chloride hydrochloride in methylene chloride at −40 °C and sparging with anhydrous ammonia for 15 min. Stirring was continued for an additional 30 min before the solution was allowed to warm to room

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temperature. Ammonium chloride and other methylene chloride-insoluble byproducts were removed by filtration through Whatman no. 1 filter paper. The methylene chloride was removed by rotary evaporation, and a pale yellow product (phenylglycine amide) was obtained. Then, 19.5 g (0.13 mol) of phenylglycine amide was added to a stirring solution of methanol (200 mL) in a three-necked round-bottomed flask. The slurry was cooled in a dry ice–methanol bath to −5 °C, and 18.8 g (0.13 mol) of 40% glyoxal was added with stirring. The reaction mixture was cooled to −20 °C and 33 mL of 20% NaOH (0.165 mol) was added in a dropwise manner. After 2 h of stirring, the solution was allowed to warm to room temperature. A clear brown solution of the sodium salt was obtained. After removal of ~125 mL of methanol by vacuum distillation, 100 mL of water was added. The solution was then neutralized by dropwise addition of 6 N HCl. At pH 9, the solution became turbid, and at pH 7, crystals of the pyrazine acid formed in the flask. After stirring the slurry overnight at room temperature at pH 7, the pH was taken to 3.0 with 6 N HCl; the slurry was stirred for an additional hour in an ice bath, and the crystals were then recovered by filtration through Whatman no. 1 filter paper. After washing with 75 mL of water at 0 °C, the crystals were air dried; 16.8 g of light brown crystals were obtained. The sample was purified by recrystallization from hot toluene, cooling to room temperature over a period of 2 h, and then cooling to 0 °C before filtration to recover the solid product. The overall yield (from phenylglycine amide) was 53.5%; ^{13}C NMR (DMSO-d_6): δ 123.07 (d, C-5), 126.77 (d, C-6), 127.74 (d, C-9, 11), 128.21 (d, C-8, 12), 129.34 (d, C-10), 136.02 (s, C-7), 151.55 (s, C-3), 155.25 (s, C-2); ^{1}H NMR: δ 7.4–7.5 [m, 5H (H-5, 6, 9, 11, 10], 8.25–8.35 [m, 2H (H-8, 12)], 12.48 [s, (br), 0.5 H (−OH].

Anal.—Calcd $C_{10}H_8N_2O$. Found 69.48, 4.76, 16.08%, C,H,N.

Compound 3—Compound 3 was produced from 3-aminomethylene-6-phenyl-piperazine-2,5-dione (3-APPD) by acidic hydrolysis. 3-APPD was prepared by degradation of cephalexin at pH 7.5 according to the procedure of Bundgaard.[11] 3-APPD readily precipitates from solution as it is formed and it was isolated by filtration. Hydrolysis of 3-APPD to 3 was accomplished as follows: 17 g (0.078 mol) of 3-APPD was added to a 2-L Erlenmeyer flask containing 600 mL of 0.2 N HCl and 1200 mL of acetone. The slurry was stirred for 6 h at 36 °C (±2 °C). After this time, essentially all the starting material had gone into solution. The slightly turbid yellow solution was filtered through Whatman no. 1 paper to remove particulates, and the acetone was removed from the filtrate by rotary evaporation. A thick crystal slurry formed during the removal of acetone, and the sample was stored in a refrigerator overnight. The crystalline product was collected by filtration and washed with 600 mL of water at 0 °C. The crystals were allowed to air dry to give 15.4 g of product; yield, 90%; ^{13}C NMR (DMSO-d_6): δ 59.1 (d, C-3), 109.33 (s, C-6), 126.61 (d, C-9, 13), 127.94 (d, C-11), 128.57 (d, C-10, 12), 135.44 (d, C-7), 139.85 (s, C-8), 160.89 (s, C-5), 163.29 (s, C-2), ^{1}H NMR: δ 4.93 [d, J = 2.4 Hz, 1H (H-3)], 6.93 [d, J = 5.4 Hz, 1H (H-7)], 7.25–7.35 [m, 3H (H-9,11,13)], 7.38 [m, 2H (H-10,12)], 8.28 [s, 1H (H-4)], 9.37 [d, J = 2.4 Hz, 1H (H-1)], 10.19 [d, J = 5.4 Hz, 1H (H-7-OH)].

Anal.—Calcd ($C_{11}H_{10}N_2O_3$) C, 60.55; H, 4.62; N, 12.84; O, 22.0. Found C, 60.31; H, 4.61; N, 12.97.

Mass Spectrometry—Fast-atom bombardment (FAB)-MS and FAB-MS/MS data were obtained with a VG ZAB-3 (B_1EB_2) triple-sector mass spectrometer or a VG ZAB-2SE (BE) two-sector mass spectrometer. Samples were dissolved in "Magic Bullet" (a 5:1 solution of dithiothreitol:dithioerythritol in methanol) and bombarded with 8 KeV Xenon atoms in the ZAB-3 or Cs ions having a net energy of 12 KeV in the ZAB-2SE. For MS/MS experiments with the ZAB-3, the precursor ions were selected by B_1 and collisionally activated (50% attenuation with He collision gas) in the second field-free region. The products were separated with a constant B_2/E linked-scan. For MS/MS experiments with the ZAB-2SE, the ions leaving the ion source were collisionally activated with helium in the first field-free region, and the products were separated by a constant B/E linked-scan.

NMR—^{1}H and ^{13}C NMR spectra were recorded on Bruker AC-250 or AM-500 spectrometers. Spectra were recorded in deuteriated dimethyl sulfoxide (DMSO-d_6) and referenced to internal tetramethylsilane unless otherwise noted. In some cases, the peaks of the initial spectrum recorded were broad and, in these cases, the addition of a trace of trifluoroacetic acid (TFA) sharpened the spectra significantly, presumably due to the conversion of a mixture of protonation states to the trifluoroacetate salts. The multiplicities of the carbon resonances were determined by the distortionless enhancement bipolarization transfer (DEPT) method.[12] Carbon–proton correlations were detected with two-dimensional heteronuclear experiments designed to detect correlations due to coupling through one[13] or more than one chemical bond.[14]

Derivatization—Derivatization with diazomethane was accomplished by generating ethereal diazomethane from 1-methyl-3-nitro-1-nitroso-guanidine (MNNG, Aldrich Chemical, Milwaukee, WI) by reaction of MNNG with NaOH and trapping of the emitted diazomethane gas with diethyl ether. Addition of excess ethereal diazomethane to <1 mg of sample dissolved in ~100 μL resulted in nearly quantitative methylation in <5 min. For acetylation derivatizations, ~100 μL of pyridine and 200 μL of acetic anhydride were added to <1 mg of sample and allowed to react overnight at room temperature before removing the solvent with a stream of nitrogen.

Results

Structure Elucidation of 5—Compound 5 eluted at a retention time of 14.78 min. The UV spectrum (λ_{max} ~243 nm) is consistent with a conjugated diene or enone. Accurate FAB-MS measurements established a molecular formula of $C_{19}H_{18}N_4O_5S_2$ (found for MH^+: m/z 447.0803; calcd: m/z 447.0797). Elemental analysis confirmed this molecular formula (calcd: C, 51.11; H, 4.06; N, 12.54; S, 14.36; found: C, 51.03; H, 4.12; N, 12.30; S, 14.57). Electron-impact MS (EI-MS) did not yield a molecular ion, presumably due to thermal instability of the molecule on the heated probe. Key ion fragments present in the EI-MS included m/z 428 ($M^{+\cdot}$ − 18), m/z 402 ($M^{+\cdot}$ − 44), and m/z 106 (Ph-CH=NH_2^+). Further MS studies were undertaken with FAB-MS/MS, and are discussed below.

Analysis of the ^{13}C NMR spectrum (Table I) confirmed the presence of 19 carbons, which can be broken into the following classes: nine sp^2-hybridized quaternary carbons; four sp^2-hybridized methines, the resonances of two of which were doubly intense (δ 128.17 and δ 129.45, corresponding to the ortho and meta carbons of a phenyl group); one sp^3-hybridized methine; and three sp^3-hybridized methylenes. These substructures account for 13 protons attached to carbon, requiring the presence of five exchangeable protons to account for the 18 protons present in the molecular formula. In the ^{1}H NMR spectrum, a broad three-proton doublet at δ 8.68 was shown to be coupled to a methine quartet at δ 5.0, suggesting the presence of a >CH-NH_3^+ group. Another exchangeable doublet resonance at δ 9.26 was shown to be coupled to a methylene group resonating at approximately δ 2.55. From a two-dimensional NMR experiment designed to correlate car-

Table I—NMR Assignments of 5 in DMSO-d_6 with a Trace of Added TFA

Site	δ, ppm	H-1 Shift(s)	Long-range Coupling
9	167.86		5.01
6	164.24		4.54
2-COOH[a]	163.22		
2′-COOH[a]	163.01		
6′	151.22	8.91	
3[b]	147.12		3.48
3′[b]	146.69		3.48
2[c]	142.72		8.91
2′[c]	141.60		
ipso	133.62		7.44
meta	129.45	7.44	
para	128.92	7.44	
ortho	128.17	7.54	
10	55.73	5.01	
7	40.65	4.54	
4[d]	28.15	3.48	
4′[d]	28.58	3.48	

[a,b,c,d] Assignments may be interchanged.

bons and protons through their two- and three-bond couplings,[14] it was possible to assemble these observations to assign the substructure 5_a. In 5_a, X represents heteroatoms. Fragments present in the mass spectra of 5 and its derivatives are supportive of the presence of substructure 5_a (from the phenyl group through the amide nitrogen; see below).

The two remaining methylene groups have very similar chemical shifts in both the ^1H and the ^{13}C NMR spectra. In a ^1H NMR spectrum measured in DMSO-d_6 without added TFA, these methylenes clearly showed a spectrum typical of an A_2B_2 spin system. A very similar pattern was also observed for the dimethyl ester derivative of this compound in pyridine-d_5. These results indicate that the protons of these methylene groups are mutually coupled, indicating that the two methylenes are connected to form an ethylene fragment. This was confirmed by long-range carbon–proton heteronuclear coupling correlations,[14] which showed that each of these methylene carbons was coupled to the protons of the other. The same experiment also showed that the protons of these methylenes were within three bonds of at least three sp^2-hybridized quaternary carbons. Therefore, the molecule was assumed to contain the substructure 5_b.

Substructures 5_a and 5_b account for all but three of the protons of the molecular formula. Two of these protons can be accounted for by the presence of two carboxylic acid groups, which were indicated by derivatization experiments (see MS discussion below). Because the carbons of these groups were shown not to be correlated to any protons through two- or three-bond coupling, these acids have no α-protons. Because the only quaternary carbons in the molecule are sp^2-hybridized, it is concluded that these acids are incorporated into a substructure like 5_c.

With these three substructures, all but six sp^2-hybridized carbons of the molecule are assigned. One of the remaining carbons is a methine with carbon and proton chemical shifts of δ 150.2 and 8.91, respectively. In a gated-decoupling ^{13}C NMR spectrum, the one-bond coupling between these nuclei was shown to be 214 Hz. These chemical shifts and coupling constants are typical for carbon-2 of thiazole.[15] Thus, a disubstituted thiazole substructure as shown in 5_d is proposed.

The UV spectrum (not shown) supports the proposal of a thiazole substructure.[15] In fact, the UV chromophore of 5 appears to be asymmetric, which would be consistent with the presence of two thiazole substructures with similar UV spectra in 5. The proposal of two thiazole moieties also explains the last three sp^2-hybridized carbon resonances of the molecule.

The logical structural possibilities for 5 can be generated with the computer program GENOA.[16] The program was given the molecular formula, the multiplicities, and hybridizations deduced from the ^{13}C NMR spectrum and the DEPT

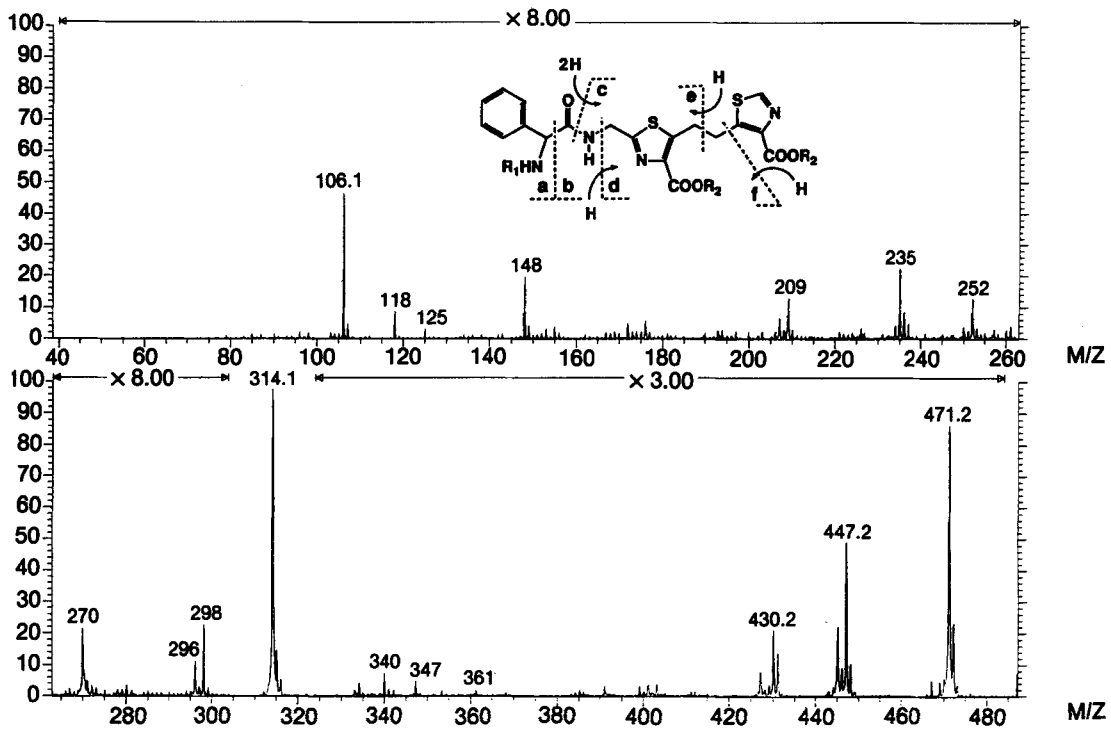
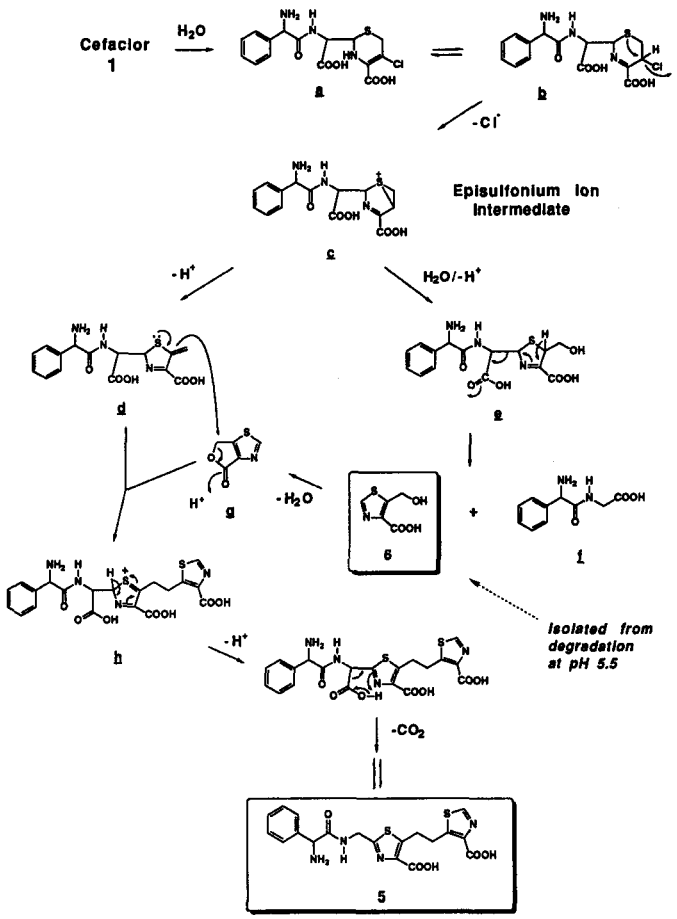


Figure 1—FAB-MS/MS of *m/z* 489, the protonated molecule of monoacetyl 5 (R_1 = Ac; R_2 = H). See Table II for a tabulation of assignments a–f for a series of derivatives of 5.

Table II—Assignment of Fragment Ions in the FAB-MS/MS of the Protonated Molecules of 5 and Its Derivatives

Compound	Ion, *m/z*[a]					
	a	b	c	d	e	f
Compound 5 (R_1=R_2=H)	106	340	314	298	305	—[b]
Dimethyl 5 (R_1=H; R_2=Me)	106	368	342	326	319	333
Monoacetyl 5 (R_1=Ac; R_2=H)	148[c]	340	314	298	347	361
Dimethyl monoacetyl 5 (R_1=Ac; R_2=Me)	148[c]	368	342	326	361	375

[a] Letters refer to the fragments shown in Figure 1. [b] Not determined. [c] Loss of ketene gives *m/z* 106.



Scheme I—Proposed formation pathway of **5**.

experiment, and the presence of one each of the substructures 5_a, 5_b, and 5_c, and two of substructure 5_d. GENOA generated 18 candidate structures for this degradation product. Four of these structures include unsaturated four-membered rings that can be considered unlikely products of the degradation conditions. Other structural features make many of the other candidates unlikely. Dramatic reduction of the number of possible candidates, however, can be achieved from a mechanistic argument. In cefaclor, the carboxylic group is attached to a carbon, which is in turn attached to nitrogen, as in substructure 5_e. It is proposed that 5_e remains intact throughout the degradation process. Addition of this constraint to the GENOA substructural list results in the removal of all structural candidates except **5**.

This structure can be supported by detailed analysis of the FAB-MS and FAB-MS/MS data. The sample was derivatized in three ways to aid in functional group detection and structure elucidation. Reaction of **5** with diazomethane increased the MH^+ from *m/z* 447 to 475 (+28 amu), indicating the presence of two -COOH groups. Reaction of **5** with acetic anhydride (Ac_2O) in pyridine yielded a product with an MH^+ of *m/z* 489 (+42 amu), which is interpreted to indicate the presence of an amino group. Finally, reaction of **5** with CH_2N_2 and then Ac_2O in pyridine increased the MH^+ to *m/z* 517 (447 + 28 + 42), confirming the presence of the functional groups deduced above. FAB-MS/MS data were obtained on **5** and these three derivatives. As an example of the data, the FAB-MS/MS spectrum of the protonated molecule of the monoacetyl derivative of **5** (*m/z* 489) is shown in Figure 1. Assignments a–f in Figure 1 are tabulated in Table II; these data were confirmed by accurate mass measurements on peaks of the same mass that were present in the FAB-MS of **5** and its derivatives. The changes induced by derivatization in fragments a–f are consistent with the assignments shown in Figure 1.

Structure Elucidation of 2—Compound **2** eluted at a retention time of 17.72 min on the analytical HPLC system. Based on comparison of the UV spectrum, FAB-MS, EI-MS, ^1H and ^{13}C NMR, and HPLC retention time of **2** to that of a synthetic standard, the structure was determined to be 2-hydroxy-3-phenylpyrazine (**2**), previously isolated from the acidic degradations of cefaclor[4] and ampicillin.[10]

Absence of 3-Formyl-6-phenyl-piperazine-2,5-dione (Diketopiperazone, 3)—Compound **3** was identified in a previous accelerated degradation study of cefaclor[4]; in this study, **3** was identified as one of the major degradation products in both acidic (0.1 N HCl, reflux for 2 h) and basic (pH 8.5, reflux for 40 min) conditions. In our study, the solution of cefaclor degrading at room temperature in 0.1 N HCl was analyzed by HPLC at several time points during the 2-month degradation. Comparison of these analyses to the HPLC analysis of a standard of **3** indicated that **3** was clearly absent (at levels >1%) from our degrading solution. We decided to assess the stability of **3** under the conditions of degradation because of the possibility of further transformation into other products. Incubation of **3** in 0.1 N HCl at room temperature for 16 days showed that the half-life of **3** is ~14 days. This instability shows that **3** is further transformed to other degradation products; however, the rate of this degradation is slow enough that were it one of the major cefaclor degradation products, it should accumulate to levels that would be easily detected. The higher temperature degradation conditions of the previous study (i.e., reflux) may be required to form appreciable amounts of this degradation product.

Analysis of the Precipitate—As cefaclor degraded, a precipitate formed in solution. The EI-MS spectrum of the precipitate revealed an $M^{+\cdot}$ of *m/z* 256 (relative intensity 4.7), with prominent fragments at *m/z* 224 (1.4), 192 (4.8), 160 (7.0), 128 (6.5), 96 (5.7), and 64 (100); the isotope peak abundances at two mass units higher than the listed peaks indicated the presence of sulfur. The spectrum indicated that the principal component of the solid was elemental sulfur (S_8).

Discussion

We have confirmed that **2**, previously identified as a degradation product of cefaclor,[4] is one of the major degradation products of cefaclor under acidic conditions. In contrast, **3**, previously identified as a degradation product of cefaclor,[4] was not detected in our study in either the precipitate or the degraded solution of cefaclor. We also identified the precipitate as primarily elemental sulfur. The formation of elemental sulfur is mechanistically intriguing; future studies of the degradation of cefaclor may shed light on the pathway.

The isolation and identification of **5** as a major degradation product of cefaclor under acidic conditions is both surprising and fascinating. Mechanistically, the formation of **5** from **1** involves a condensation of two cefaclor degradation products, in which both products have undergone a six-to-five-membered ring contraction. A proposed mechanism is outlined in Scheme I.

The formation of **5** from cefaclor probably begins with the opening of the β-lactam ring to form intermediate **a**. Tautomeric shift of the double bond should favor the imine from **b**. A 1,3-transannular attack of sulfur on C3 would eliminate chloride to form episulfonium ion **c**, which appears to be central to the degradation of cefaclor under acidic conditions.[17] One possible reaction of **c** involves the loss of a proton to form **d**. Another possible reaction is the attack of water to form intermediate **e**, which could fragment upon aromatiza-

tion to form **6** and **f**. It should be noted that **6** has been isolated from an aqueous degradation of cefaclor at pH 5.5.[17] Lactonization of **6** yields an alkylating agent (**g**) that can react with **d** to form **h** which, upon deprotonation, aromatizes to **i**. Subsequent acid-catalyzed decarboxylation of **i** leads to **5**.

Six-to-five-membered ring contractions involving episulfonium intermediates have been previously observed in synthetic interconversions of penam and cepham systems.[18] We believe the formation of **5** and **6** from **1** are the first reported examples of such a ring contraction resulting from the aqueous degradation of cephalosporins. That **5** was the most abundant degradation product under the conditions studied suggests the facility of this pathway, as well as the possibility that the generality of this rearrangement may extend to other cephalosporins containing leaving groups at the 3-position.

References and Notes

1. Knapp, C. C.; Washington, J. A. *Antimicrob. Agents Chemother.* **1988**, *32*, 131–133.
2. Cefaclor has a half-life of ~24 h at 25 °C, pH 6, in 0.1 M potassium phosphate buffer, and a half-life of ~2 h at 37 °C in human serum or plasma (see Foglesong, M. A.; Lamb, J. W.; Dietz, J. V. *Antimicrob. Agents Chemother.* **1978**, *13*, 49–52).
3. Dinner, A. *J. Med. Chem.* **1977**, *20*, 963.
4. Compound **3** was isolated from both an accelerated acidic degradation (0.1 N HCl, reflux for 2 h) and an accelerated basic degradation of cefaclor (pH 8.5, ammonium hydroxide buffer, reflux for 40 min); **2** was isolated only from the accelerated acidic degradation; Tai, J., unpublished data, Eli Lilly and Company, Lilly Research Laboratories, Indianapolis, IN.
5. Indelicato, J. M.; Dinner, A.; Peters, L. R.; Wilham, W. L. *J. Med. Chem.* **1977**, *20*, 961–963.
6. Nakashima, E.; Tsuji, A.; Nakamura, M.; Yamana, T. *Chem. Pharm. Bull.* **1985**, *33*, 2098–2106.
7. Lorenz, L. J. In *Analytical Profiles of Drug Substances*, Vol. 9; Florey, K., Ed.; Academic Press: New York, 1980, pp 117–118.
8. Sides, G. D.; Franson, T. R.; DeSante, K. A.; Black, H. R. *Clin. Ther.* **1988**, *11*, 5–17.
9. Jones, R. G. *J. Am. Chem. Soc.* **1949**, *71*, 78.
10. Lebelle, M. J.; Vilim, A.; Wilson, W. L. *J. Pharm. Pharmacol.* **1979**, *31*, 441–443.
11. Bundgaard, H. *Arch. Pharm. Chemi. Sci. Ed.* **1977**, *5*, 149–155.
12. Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Reson.* **1982**, *48*, 323.
13. Derome, A. E. In *Modern NMR Techniques for Chemistry Research*; Baldwin, J. E., Ed.; Pergamon: New York, 1987; Chapter 9.
14. Martin, G. E.; Zektzer, A. S. *Magn. Reson. Chem.* **1988**, *26*, 640–641.
15. Metzger, J. V.; Vincent, E.-J.; Chouteau, J.; Mille, G. In *Thiazole and Its Derivatives*; Metzger, J. V., Ed.; Wiley Interscience: New York, 1979; pp 5–149.
16. GENOA is a computer program designed to generate all candidate structures consistent with the molecular formula and a given set of substructural constraints (cf. Carhart, R. E.; Smith, D. H.; Gray, N. A. B.; Nourse, J. G.; Djerassi, C. J. *Org. Chem.* **1981**, *46*, 1708–1718).
17. Baertschi, S. W.; Dorman, D. E.; Occolowitz, J. L.; Spangle, L. A.; Collins, M. W.; Lorenz, L. J., unpublished results.
18. Kukolja, S.; Lammert, S. R.; Gleissner, M. R.; Ellis, A. I. *J. Am. Chem. Soc.* **1975**, *97*, 3192–3198.

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