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Isolation and Structure Elucidation of the Major Degradation Products of Cefaclor in the Solid State

DOUGLAS E. DORMAN[x], LESLIE J. LORENZ, JOHN L. OCCOLOWITZ, LARRY A. SPANGLE, MONTE W. COLLINS, FADIA N. BASHORE, AND STEVEN W. BAERTSCHI

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Abstract □ Cefaclor is a β-lactam antibiotic that degrades slowly under normal storage conditions to several minor products. To obtain samples large enough to permit structure elucidation, cefaclor was allowed to degrade at 40 °C (75% relative humidity) and at 85 °C. The profile of degradation products formed under these conditions is qualitatively similar to the profile of degradation products observed in samples of cefaclor aged for 14 years at room temperature, although some products found in the sample degraded at 85 °C are not formed at the lower temperatures. Using preparative reversed-phase high-performance liquid chromatography (rp-HPLC) and a combination of spectroscopic methods, we have isolated and characterized 17 of these degradation products. Some of these products were also isolated from studies of aqueous degradations. The major products appear to have arisen from five distinct pathways: (1) isomerization of the double bond in the dihydrothiazine ring; (2) decarboxylation; (3) ring contraction of the cephem nucleus to thiazole structures; (4) oxidative attack at carbon 4 of the dihydrothiazine ring; and (5) intramolecular attack of the primary amine of the side chain on either the β-lactam carbonyl to form 3-phenyl-2,5-diketopiperazines or the "masked aldehyde" at carbon 6 to form 2-hydroxy-3-phenylpyrazine derivatives. The pathway involving oxidation at carbon 4 is particularly important at ambient temperatures and is unique to the solid-state degradation.

Introduction

Cefaclor (**1**) is a cephalosporin antibiotic that is administered orally in the form of both aqueous solutions and solid dosages. Cefaclor degrades under aqueous conditions to many compounds, some of which have been characterized and found to arise from only a few distinct pathways.[1,2] The degradation pathways of cefaclor in the solid state might be expected to differ from those observed in solution, but these pathways have not yet been delineated.

Previous research on the solid-state stability of cefaclor indicates that it degrades slowly to numerous products.[3] Clearly, obtaining samples large enough for complete characterization in a reasonable length of time requires more vigorous degradation conditions. In this report we evaluate the degradation of cefaclor in the solid state under the following conditions: (**A**) 13 months at 85 °C; (**B**) 14 years at room temperature; and (**C**) 3 months at 40 °C and 75% relative humidity (RH). The profiles of degradation products obtained under these conditions are comparable, although there are some unique degradation products in sample **A** (see Figure 1).

Experimental Section

Materials—Cefaclor bulk drug substance (defined as the monohydrate, crystalline) was obtained from production lots of cefaclor.

Solid-State Degradations—Cefaclor was stored in the solid state under the following conditions: (**A**) 13 months at 85 °C; (**B**) 14 years at room temperature; and (**C**) 3 months at 40 °C and 75% RH. All samples were stored in containers with closures that permitted passage of atmospheric gases. These samples of cefaclor were analyzed for potency by a recently developed method (see Figure 1).[3] The analyses showed the potency of the cefaclor to be 5% from **A**, 69% from **B**, and 84% from **C**.

Analysis by rp-HPLC—The analytical scale rp-HPLC system utilized a photodiode array detector [deuterium lamp; wavelength accuracy ±1.0 nm adjusted to the line spectrum of the deuterium lamp at 656.1 nm; ultraviolet (UV) detection from 200−400 nm at 4 nm resolution and a 1-s interval between acquisition of successive spectra; Waters model 991; Waters, Milford, MA] to detect eluting peaks. The sample was injected with the system equilibrated and pumping 100% solvent A [solvent A was prepared by dissolving $NaH_2PO_4 \cdot H_2O$ (6.9 g/L) and adjusting the pH to 6 with 5 N NaOH]. After 10 min, solvent B [solvent B was a mixture of 30% methanol, 20% acetonitrile, and 50% solvent A (by volume)] composition was increased to 25% at a rate of 4% per min, and held at 25% B for an additional 10 min. Solvent B was then increased to 100% at a rate of 4% per min and held at 100% for 10 min. The system was equilibrated for 10 min at 100% solvent A prior to each injection. The flow rate was 1.0 mL/min. The column used was a YMC-Basic (4.6 × 250 mm, B-03−5, YMC, Morris Plains, NJ).

Isolation of Degradation Product—The cefaclor bulk degraded at 85°C was dissolved in water at a concentration of 5 mg/mL and filtered prior to fractionation by preparative rp-HPLC. The individual degradation products were isolated from the aqueous solution using HPLC, rotary evaporation, and lyophilization in the same manner as described previously.[2] A total of 117 10-mL preparative injections were made.

Identification of Compounds of Known Structures—Authentic benzamide (**20**) was obtained from Aldrich Chemical Company, Milwaukee, WI. 5-Phenylhydantoin (**21**) was obtained from TCI America, Inc., Portland, OR. The preparations of **14**, **5**, and **6** are published in our accompanying paper.[2]

Measurement of Spectra—All spectra were measured by the methods described in our accompanying paper.[2]

Results

Comparison of Solid-State Degradations under Various Conditions—Stressed cefaclor samples **A**, **B**, and **C** were analyzed by gradient rp-HPLC by UV-photodiode array detection. This analysis revealed chromatograms that are qualitatively similar (Figure 1). Many of the peaks with retention times of 42 to 55 min are associated with UV spectra characteristic of substituted 2-hydroxy-3-phenylpyrazines.[2] Thus, the formation of substituted pyrazines that occurs under acidic aqueous conditions also occurs during solid-state degradation, although to a much smaller extent. Analysis of the chromatograms and UV spectra revealed that most of the degradation products present in the sample of cefaclor stressed at 40 °C and 75% RH and in the sample aged at room temperature for 14 years are present in the 85 °C-stressed sample. For this reason, the 85 °C-stressed sample was used to provide larger quantities of solid-state degradation products for isolation by preparative HPLC (see *Experimental Section*).

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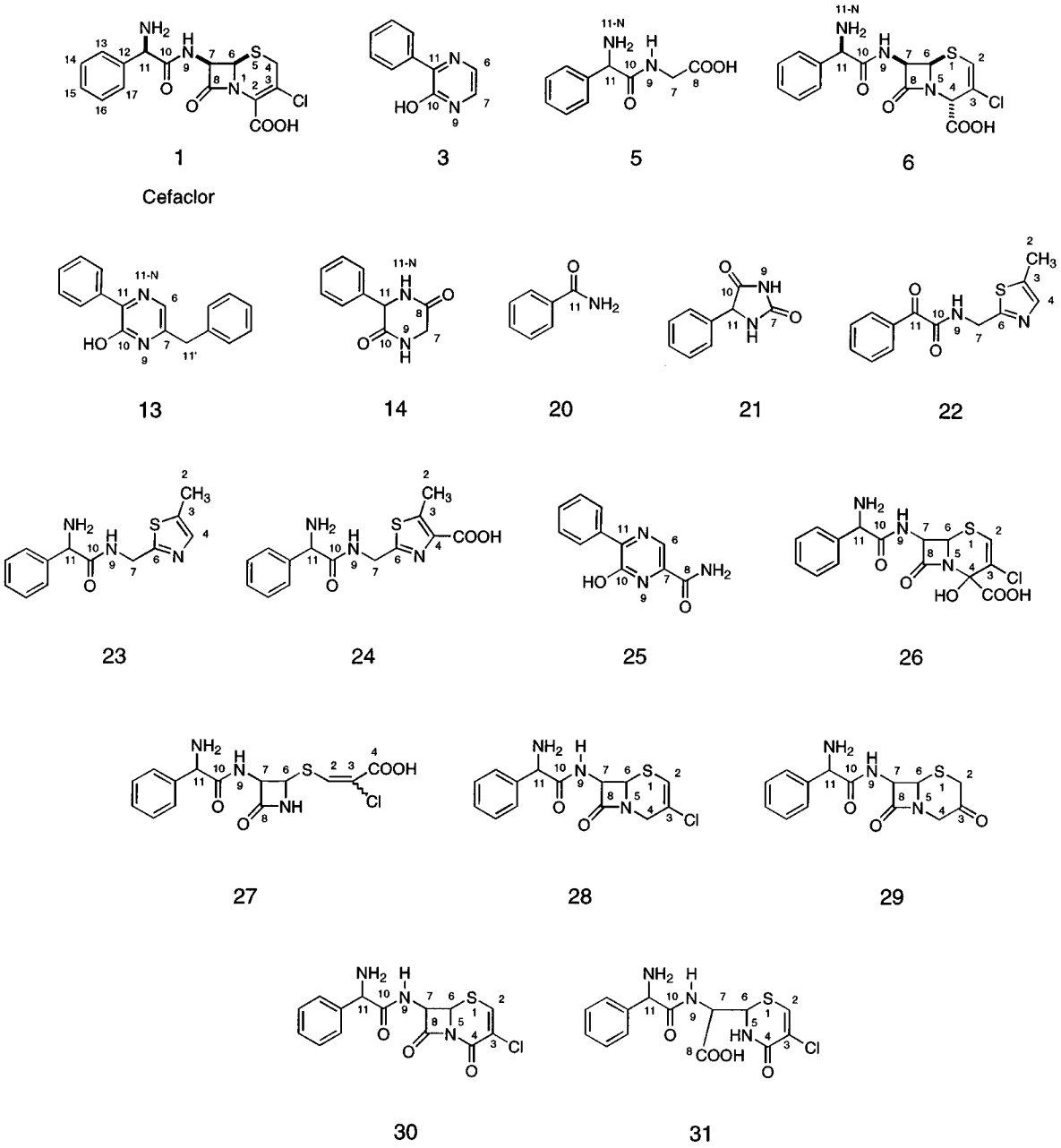
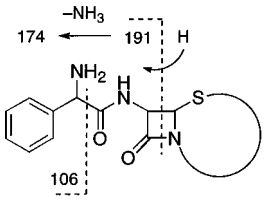


Chart 1—Structures of cefaclor and solid-state degradation products.

Elucidation of Structures—Because of the structural relationships between the compounds isolated from these degradations, it was possible to identify some generalities in their properties and spectra that were useful in structure elucidation. For example, those compounds with the phenylglycyl substructure have a peak at m/z 106 in the mass spectra. Other characteristics of thiazole, pyrazine, and other substructures are described in our accompanying paper.[2]

Among the products of the present degradation, however, were seven compounds in which the fundamental structure of the parent antibiotic survived. In five of these compounds, the β-lactam was intact, as shown by the presence of a peak near 1770 cm^{-1} in the infrared (IR) spectrum. In these cases, the degradation products gave collision-induced fast-atom bombardment-mass spectroscopy/mass spectroscopy (FAB-MS/MS) fragment ions in the mass spectra at m/z 191 and 174. These fragment ions are also observed in the spectra of cefaclor and Δ^2-cefaclor (**6**), and result from initial cleavage through the β-lactam ring, followed by elimination of NH_3 from the phenylglycyl moiety:



In those cases in which there was sufficient compound for NMR spectroscopy, the three-spin pattern of the H6, H7, and NH resonances[4] also confirmed the presence of the β-lactam substructure in these products.

For the purpose of NMR resonance assignment, the sites of the structures discussed in this paper are numbered on the basis of their proposed mechanistic origins from cefaclor. This numbering permits tabulation of the assigned data in a way

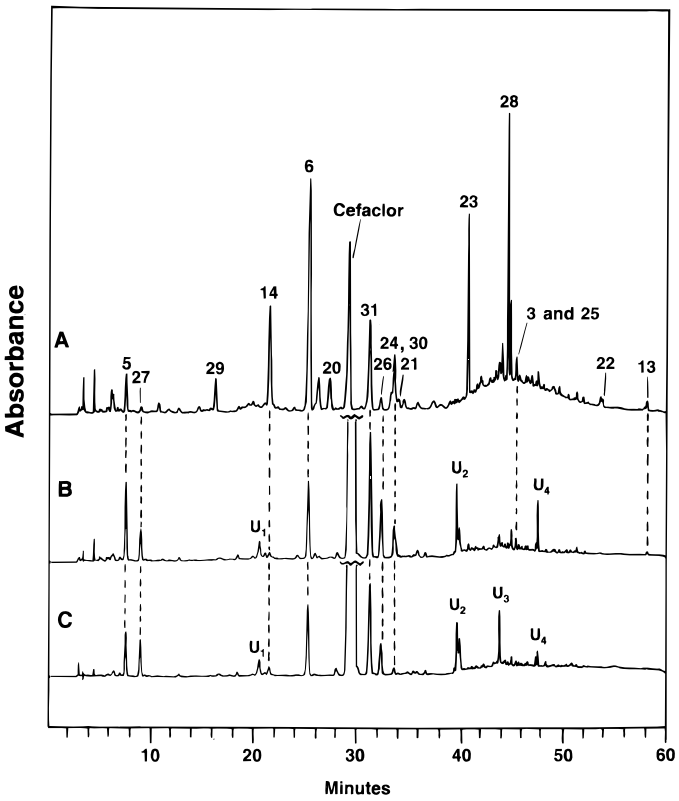
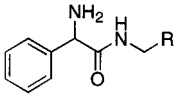


Figure 1—rp-HPLC chromatograms (UV, 220 nm) of partially degraded bulk cefaclor samples: (A) stored for 13 months at 85 °C; (B) stored for 14 years at room temperature; (C) stored for 3 months at 40 °C and 75% RH.

that emphasizes any existing structural similarities (*e.g.*, see Table 1). In the discussion that follows, the 17 degradation products isolated in this research are subdivided into five categories: thiazoles (**22**, **23**, **24**); pyrazines (**3**, **13**, **25**), also referred to as "fluorescent products"; compounds resulting from C_4-oxidation (**26**, **27**, **30**, **31**); simple hydrolysis products (**14**, **5**); and miscellaneous structures (**20**, **21**, **6**, **28**, **29**).

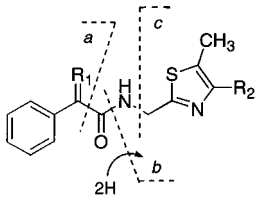
Thiazole-Containing Products—Three of the isolated degradation products, **22**, **23**, and **24**, contain the thiazole substructure. Although all three of these products are observed in sample **A**, only compound **24** was detected in the other two samples (Figure 1).

*Compound **23***—Compound **23** is one of the major products observed when cefaclor was degraded at 85 °C (see sample **A** of Figure 1). The UV spectrum shows a λ_{max} of 250 nm (see Figure 2). Based on high-resolution FAB-MS, the molecular formula of **23** is $C_{13}H_{15}N_3OS$. Fragment ions in the MS spectrum are consistent with the presence of a phenylglycyl group, a conclusion that is confirmed by the NMR spectra (Table 1). From the ^{1}H NMR spectrum, it can be deduced that there is an amide NH proton (δ 9.32) that is vicinally coupled to a methylene group with protons coming into resonance at δ 4.59 and 4.52, and from the heteronuclear multiple bond correlation (HMBC) experiment,[5] which correlates carbon and proton resonances through their two- and three-bond coupling constants, it is clear that this group is attached to the phenylglycyl moiety:



The NMR spectra also include resonances of a methyl group, and from the carbon and proton chemical shifts and absence

Table 1—Spectroscopic Data for the Thiazole Degradation Products



Parameter	Degradation Product 22	23	24
Mol. Form	$C_{13}H_{12}N_2O_2S$	$C_{13}H_{15}N_3OS$	$C_{14}H_{15}N_3O_3S$
MH^+ found	261.0703	262.0986	306.0897
MH^+ calc	261.0698	262.1014	306.0912
UV	257	250	243
fragment *a*[a]	105	106	106
fragment *b*	129	129	173
fragment *c*	112	112	156
2	11.55/2.43	11.56/2.37	12.78/2.61
3	134.17	134.50	141.3[b]
4	139.96/7.43	139.68/7.39	144.14[b]/–
4′	---	---	163.24[c]
6	165.14	166.27	163.32[c]
7	40.18/4.67	40.77/4.59,4.52	40.51/4.52
9	9.75	9.32	9.35
10	165.72	167.94	167.86
11	189.93/–	55.98.5.04	55.64/5.02
11-N	---	8.74	8.74
12	132.72	134.00	133.68
13,17	129.84/8.02	128.38/7.56	128.11/7.5
14,16	129.05/7.60	129.10/7.46	128.93/7.5
15	134.80/7.76	129.60/7.46	129.4/7.5

[a] Fragments defined by the general structure above. [b] Resonances so designated can be reversed in assignment.

of vicinal coupling in the ^{1}H NMR spectrum, it is clear that this methyl is attached to an unsaturated quaternary carbon. One of the two remaining resonances in the ^{13}C NMR spectrum is that of an unsaturated methine, the carbon (δ 139.68) and proton (δ 7.39) of which are both coupled to the protons of the methyl group. This situation requires that the molecule contains the following substructure:

These substructures account for all of the protons and all but one of the carbons of the molecular formula. The last carbon is represented in the ^{13}C NMR spectrum by a resonance at δ 166.27 that is correlated through two- or three-bond couplings to the protons of both the methylene group and the methine of the substructures just described.

These substructural deductions, combined with the assumption of a thiazole ring, are shown by GENOA[6] to be consistent with two structures that differ only in the placement of the methyl on the thiazole ring. Of these, we chose **23** on the basis of the following mechanistic arguments. As we have previously shown,[1] the thiazole products arise from ring contraction through an intermediary episulfonium ion.[7] This ring contraction leaves the methyl attached to the carbon adjacent to the sulfur atom. Subsequent decarboxylations and aromatization of this intermediate yield **23**. Additional confirmatory spectroscopic data collected from this compound are presented in Table 1.

*Compound **22***—As shown in Figure 1, **22** is a late-eluting component of sample **A**. The UV spectrum (λ_{max} 257 nm) suggests that it contains a thiazole substructure (see Table 1

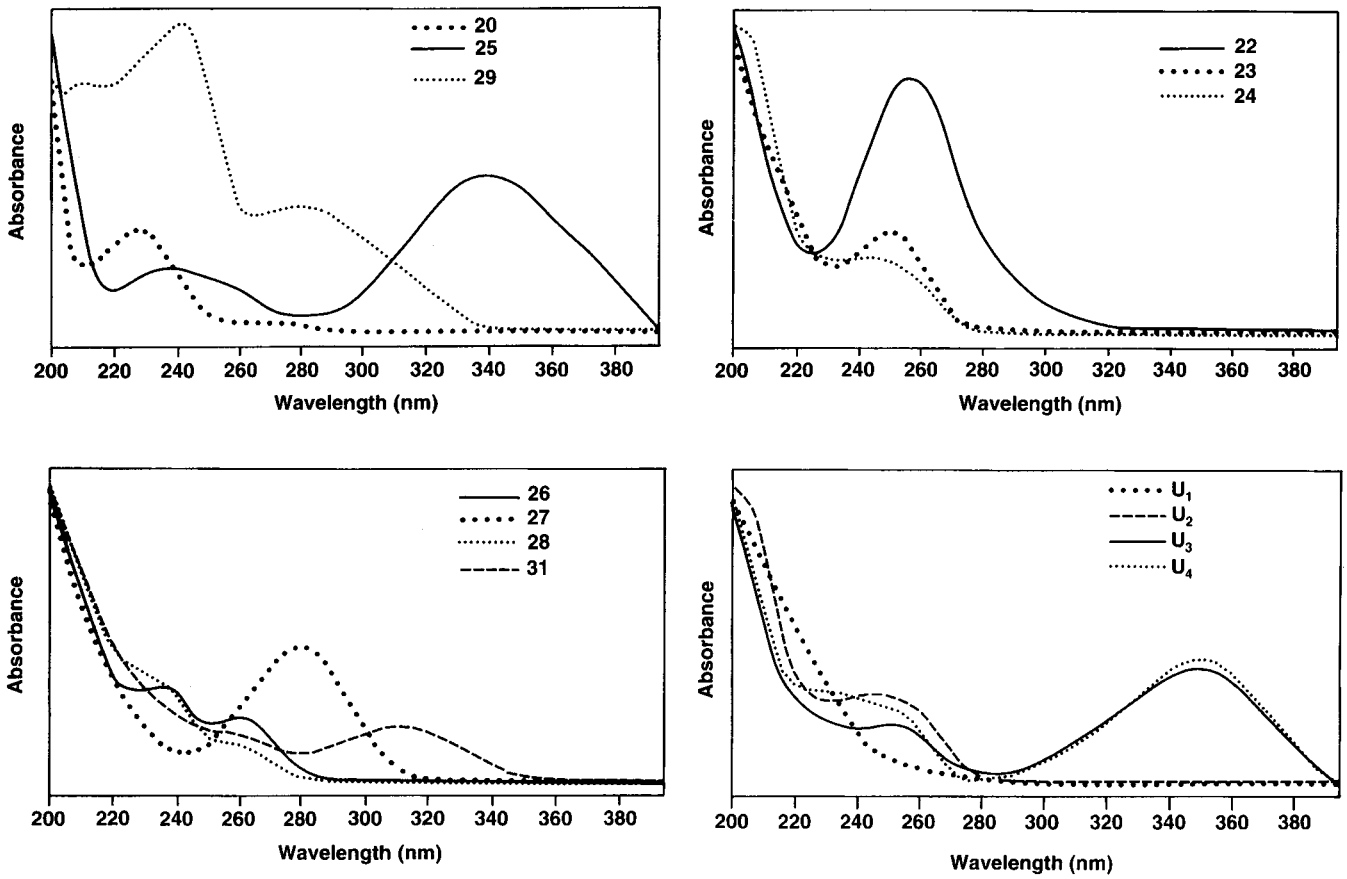


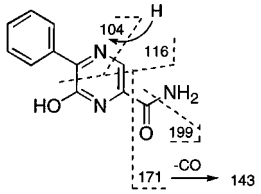
Figure 2—UV spectra of some of the degradation products obtained from photodiode array UV detector as the compounds eluted from the column.

and Figure 2). The molecular formula of **22** ($C_{13}H_{12}N_2O_2S$) differs from that of **23** by the replacement of a nitrogen and three protons by a single oxygen atom, which suggests that **22** could be derived from **23** by oxidative deamination. This proposal is confirmed by the mass spectrum, in which the usual m/z 106 peak has been replaced by a peak at m/z 105, which was shown by its accurate mass measurement to be a benzoyl ion (found, 105.0341; calc for C_7H_5O, 105.0340). Except for this difference, the mass spectrum of **22** was very similar to that of **23**. In the NMR spectra (Table 1) there are close similarities in the chemical shifts of **23** and **22** in the thiazole half of the molecule. The aromatic phenyl region of the NMR spectrum of **22**, however, is more typical of a phenyl substituted by an electron-withdrawing group,[8] and the usual C11 methine carbon and proton resonances associated with the phenylglycyl substructure (*vide supra*) have been replaced by a new carbonyl resonance at δ 189.93. The structure of this compound is therefore proposed to be **22**.

*Compound **24***—Compound **24**, formed in all three of the degradation conditions studied (see Figure 1), has a UV spectrum consistent with a thiazole chromophore (λ_{max} 243 nm, see Table 1 and Figure 2). Compound **24** was shown by accurate mass FAB-MS measurements to have a molecular weight of 305 ($C_{14}H_{15}N_3O_3S$). The molecular formula of **24** compared with that of **23** (Table 1) differs by the elements of carbon dioxide. In the mass spectra, the fragment ions *b* and *c* (see figure at top of Table 1) are shifted 44 amu to higher mass, showing that the carboxylate group must be attached either to the methylene or the thiazole ring. Because there are clear indications of a methylene group in the NMR spectra (δ 40.51 and 4.52, carbon and proton chemical shifts, respectively), **24** was proposed. This structure is confirmed by the absence of an aromatic methine in the ^{13}C NMR spectrum, and by chemical shift changes relative to **23** localized in the thiazole substructure (see Table 1).

"Fluorescent Products"—Three of the isolated products from the bulk degradations included the pyrazine substructure, as indicated by the UV spectrum (**3**, **13**, and **25**). Two of these (**3** and **13**) were previously isolated from acidic degradations.[2] Spectroscopic data for these compounds are presented in Table 2.

*Compound **25***—This compound was shown by MS to have the molecular formula $C_{11}H_9N_3O_2$, just CHNO larger than 2-hydroxy-3-phenylpyrazine (**3**). The UV spectrum was similar to that of 2-hydroxy-3-phenylpyrazine. From these data it was proposed that **25** was a derivative formed from addition of a carboxamide or aldoxime group to the pyrazine ring. A fragment ion characteristic of the loss of NH_2 in the MS/MS spectrum led to the choice of the carboxamide alternative, **25**. Other fragmentations supporting this structure are shown here.



The ^1H NMR spectrum includes the resonances of three exchangeable protons (δ 12.19, 8.01, and 7.91), which is also consistent only with the carboxamide proposal. To confirm

Table 2—NMR Data for the Fluorescent Products[a]

Site	Product[b]		
	3 ($C_{10}H_8N_2O$)	**13** ($C_{17}H_{17}N_2O$)	**25** ($C_{11}H_9N_3O_2$)
6	126.80	122.60	128.84
	7.40	7.43	7.47
7	123.09	140.43	n.o.
	7.4	–	–
8	–		174.63
9	–	–	8.01,7.91
10	155.28	155.85	n.o.
	–	–	–
10-OH	12.68	≈12.6	12.19
11	151.57	148.30	n.o.
	–	–	–
12	136.06	136.08	135.63
	–	–	–
13,17	128.24	128.16	128.08
	8.31	8.30	8.27
14,16	127.76	127.82	128.84
	7.44	7.43	7.48
15	129.36	129.13	129.97
	7.44	7.40	7.48

[a] n.o., Not observed due to the low sensitivity of the ^{13}C NMR experiment. [b] Molecular formula given in parentheses.

Table 3—NMR Spectra of Degradation Products Resulting from Oxidation

Site	Product			
	6	**26**	**27**	**31**
1		---	9.02	---
2	118.23	119.28	139.89	128.85[a]
	6.75	6.84	7.28	8.13
3	113.83	121.85	113.40	119.09[a]
4	54.02	77.16	163.59	158.26[a]
	4.87			
4-COOH	167.28	166.44	---	---
6	52.64	53.12	60.72	55.59[a,b]
	5.18	5.16	5.26	4.82
7	59.94	59.32	61.95	55.04[a,b]
	5.62	5.57	5.39	4.82
8	163.37	160.38	165.05	169.59[a]
9	9.64	9.78	9.59	9.22
10	168.09	168.31	167.86	168.04[a]
11	55.50	55.29	55.59	56.72[a,b]
	5.06	5.07	5.01	5.08
11-N	8.84	8.74	8.71	8.70
12	133.53	133.44	133.17	133.36[a]
13,17	127.94	127.85	127.67	128.85[a]
	7.54	7.53	7.47	7.44
14,16	128.88	128.85	128.56	128.51[a]
	7.44	7.46	7.34	7.44
15	129.44	129.44	129.13	129.32[a]
	7.44	7.46	7.34	7.44

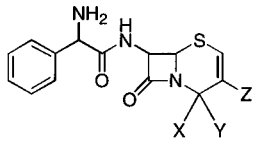
[a] Peaks so marked are doubled. [b] Assignments may be interchanged.

that these resonances were assignable to exchangeable sites, the temperature of the sample was raised and the ^1H NMR spectrum was remeasured. This procedure led to the expected large changes[9] in the resonances of the exchangeable protons, and to smaller shifts of those of aromatic protons. These chemical shift changes fortuitously led to the resolution of previously overlapped resonances, so that three groups of aromatic protons could be identified. Two of these groups (δ 8.26 and 7.47) were the pattern of resonances typical of the 2-hydroxy-3-phenylpyrazine system.[2] The third group was a singlet resonance at δ 8.30, which can be assigned to the remaining pyrazine proton (H6). The specific location of the carboxamide on the pyrazine ring was proposed on the basis of mechanistic arguments.[10] The appearance of a fragment at m/z 116 (see structure just shown) provides additional evidence for this assignment.

Products Resulting from C_4 Oxidation—Four of the degradation products isolated during this research (**26**, **27**, **30**, and **31**) were shown to result from oxidation at carbon 4. These structures were unlike any products isolated from degradations in solution. Hence, the processes leading to these compounds are unique to the solid-state degradations of cefaclor. The NMR data for these compounds appear in Table 3.

Compound ***26***—This compound was shown by mass spectrometry to have the molecular formula $C_{15}H_{14}N_3O_5SCl$, which suggests that it results from oxygenation of cefaclor (Table 3). The mass spectrum also included fragment ions indicating that the phenylglycyl and β-lactam units are intact, a conclusion supported by IR and NMR spectroscopies. Hence it was concluded that this product differed from cefaclor by oxidation of the thiazine ring.

Two of the degradation products isolated in this research (**6** and **28**) have substructures in which the double bond of the thiazine ring has migrated to the 2,3-position. The UV spectra of these compounds include peaks or shoulders at ~240 and 260 nm (see Figure 2). The UV spectrum of **26** clearly falls into this type (see Figure 2) and we therefore propose the following partial structure:



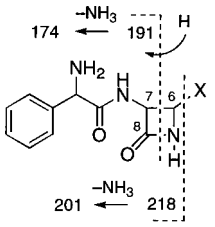
In support of this proposal, the NMR data indicate the presence of an sp^2-hybridized methine (δ_C/δ_H: 116.85/6.56; see Table 3) in the molecule, and the HMBC experiment shows that the resonance of carbon 6 is correlated through three-bond coupling with this vinyl proton. Also, long-range coupled to this proton are the resonances of two quaternary carbons, one at δ 125.83 (C3) and another at δ 77.79 (C4). If we make the reasonable assumption that Z = Cl and Y = COOH, then X can only be OH, and the structure must be **26**.

Comparison of the NMR spectra of **6** and **26** confirms this conclusion (Table 3). For example, the chemical shift of carbon 4 in **26** is >23 ppm downfield relative to the same site in **6**, consistent with oxygen substitution in the former. Significant chemical shift changes are also seen at sites 2, 3, and 8.

Compound ***27***—Although many of the spectroscopic properties of this degradation product are similar to those of the other products, there are unusual features. Thus, although the IR spectrum indicates that the compound contains a β-lactam, the peak usually assigned to this substructure is split (1772 and 1746 cm^{-1}). The FAB-MS/MS mass spectrum of the MH^+ ion has the usual peaks at m/z 106, 174, 191, and 218, but in the case of **27**, the m/z 218 peak is the largest of the spectrum rather than one of the less intense peaks as in other examples. In fact, the m/z 218 fragment ion is so prevalent that a fragment (m/z 201) derived from it by loss of NH_3 leads to the second most intense peak in the spectrum. In the UV spectrum there is absorption at 280 nm.

Unusual features are also present in the NMR spectra. While the NMR spectra look much like those of other β-lactam compounds, but they indicate the presence of two amide NH groups instead of the usual one, and *both* of these NH protons are coupled to H7! The first of these resonances is a doublet (J = 8.3 Hz) at δ 9.56, and the other is a doublet with an

unusually small coupling (1.3 Hz) at δ 9.01. In the HMBC spectrum,[5] both amide NH protons are correlated to the β-lactam carbonyl and to carbon 6. One way to explain all these results is to propose the following partial structure:

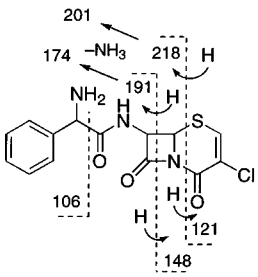


An obvious advantage of this structure is that it provides an explanation for the facile cleavage between X and carbon 6 to lead to the intense fragment observed at *m/z* 218.

There remain three carbons, two protons, two oxygen atoms, and one each of chlorine and sulfur to account for. The NMR data show the presence of one additional trisubstituted double bond. The vinyl proton of this double bond is shown by the HMBC experiment to be within three bonds of carbon 6 and of a carbonyl carbon coming into resonance at δ 162.68. These data can be accounted for by structure **27**.

In the initial ^{1}H NMR spectrum, the resonance later assigned to H2 came into resonance at δ 7.15. The measurement of the carbon and heteronuclear correlation experiments were delayed for 3 days. During this time, the position of the resonance of this vinyl proton changed to δ 7.23. Remeasurement of the ^{1}H NMR spectrum confirmed that this resonance had indeed shifted, whereas no other resonance in the spectrum had undergone significant change. This chemical shift change apparently results from isomerization of the double bond. The initial product must be the E-isomer, with the sulfur and chlorine atoms *trans* to each other. With time, this *E*-isomer may isomerize to the Z-isomer. The presence of both isomers may explain the doubling of the β-lactam peak in the IR spectra just noted. We do not presently have spectroscopic data that allow a definitive solution for this structural detail.

*Compound **30***—Compound **30** was studied as an impurity in the fraction containing **20** (benzamide), and thus its proposed structure depends almost entirely on accurate mass and MS/MS data derived from the MH^+ ion. Comparison of the molecular formula of this compound ($C_{14}H_{12}N_3O_3SCl$) with that of cefaclor ($C_{15}H_{14}N_3O_4SCl$) shows that these two compounds differ by the elements of formaldehyde. In the MS/MS sprectrum of the protonated molecular ion there was the *m/z* 106 peak typical of the presence of phenylglycyl, and the *m/z* 191 and 218 peaks were complemented by peaks at *m/z* 148 and 121, respectively, both of which were shown by their isotope patterns to contain chlorine. Therefore, this product was proposed to be **30** and the results of the MS/MS spectrum are explained as shown in the following structure:



In support of this proposal, HPLC analysis of the fraction including this compound revealed a peak with UV spectrum (not shown) essentially identical to that of **31** (see Figure 2).

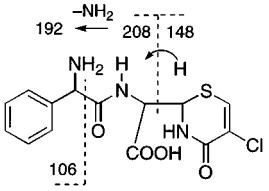
Table 4—NMR Chemical Shifts of Miscellaneous Degradation Products

Site	Product **20**	**21**	**6**	**28**[a]	**29**
2	–	–	118.23	115.21	33.02
	–	–	6.75	6.58	4.36,3.91
3	–	–	113.83	116.26	199.65
4	–	–	54.02	41.21	49.61
	–	–	4.87	4.21,3.77	3.64,2.96
4′	–	–	167.28	---	---
6	–	–	52.64	59.42[b]	60.35
			5.18	5.10	5.63
7	–	157.53	59.94	56.32[b]	57.17
		–	5.62	5.54	5.47
8	–	–	163.37	164.26[c]	166.0-
9	–	10.77[b]	9.64	9.62	–
					9.63
10	–	174.24	168.09	170.22[c]	168.28
11	167.93	61.22	55.50	53.62	55.82
	–	5.16	5.06	5.05	5.10
11-N	7.97,7.36	8.39[b]	8.84	8.77	–
					8.7
12	134.33	136.10	133.53	136.46	133.89
13,17	128.06[a]	126.74[c]	127.94	127.49	128.29
	7.88	7.23	7.54	7.5	7.5
14,16	128.24[a]	128.69[c]	128.88	128.56	129.02
	7.45	7.23	7.44	7.5	7.5
15	131.26	128.29	129.44	128.56	129.5
	7.52	7.23	7.44	7.5	7.5

[a] Carbon NMR spectrum measured in DMSO-d_6 without added TFA; therefore, chemical shifts do not reflect complete protonation of the 11-NH_2 group; Proton chemical shifts were measured after addition of TFA. [b] Resonances so marked and within a single column may be interchanged in assignment.

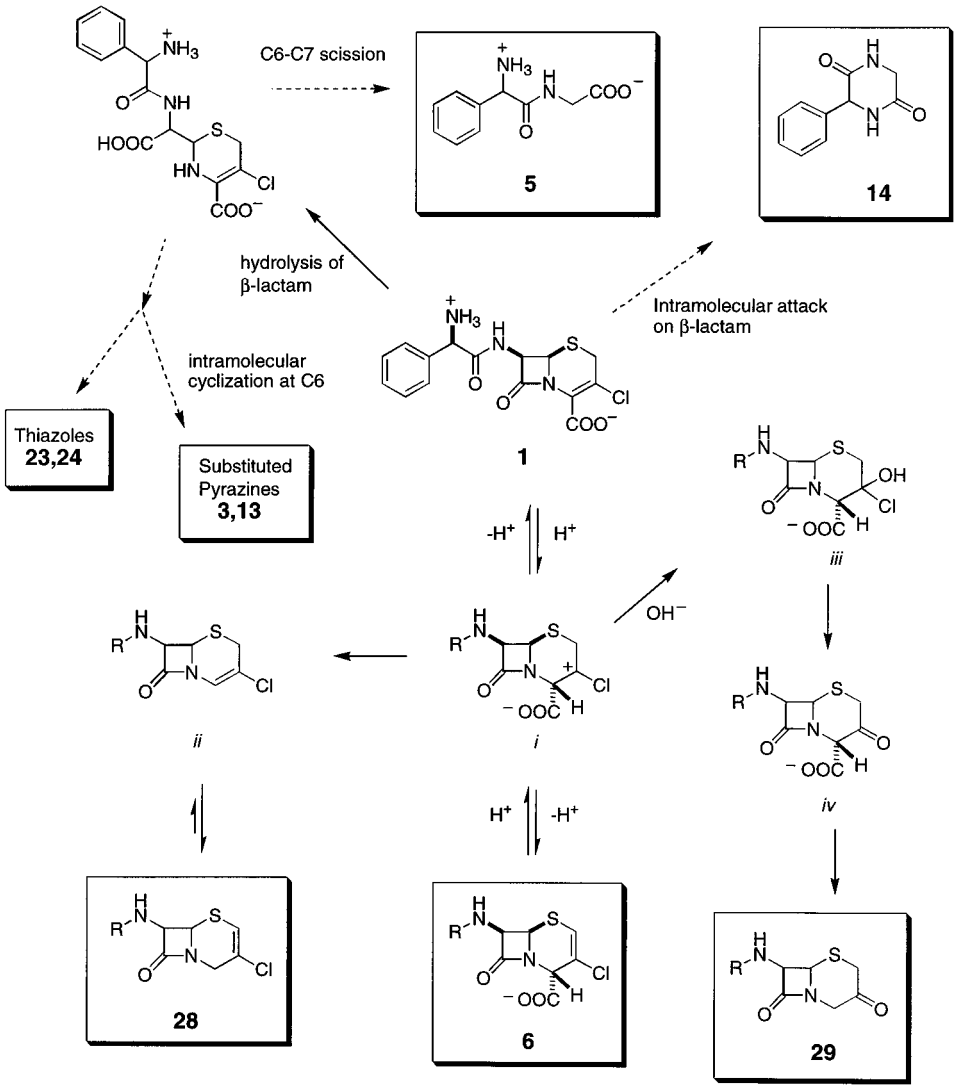
Finally, the pathways of oxidative degradation proposed next necessitate the presence of such an intermediate in the reaction medium.

*Compound **31***—From the molecular formula ($C_{14}H_{14}N_3O_4SCl$) it is seen that **31** is simply one molecule of water larger than **30**, and it can therefore be proposed that this product results from opening the β-lactam ring of the latter compound. In confirmation of this proposal, the IR spectrum does not include a typical β-lactam peak. In addition, the *m/z* 191 peak generally observed in the mass spectra of the β-lactams (already shown) is shifted by 17 amu to *m/z* 208. This fragment loses NH_2 to form the derivative ion *m/z* 192. A chlorine-containing fragment at *m/z* 148 complements the *m/z* 208 fragment. Reaction of the compound with acidic methanol leads to the formation of a methyl ester, the MS/MS of the MH^+ ion of which showed the expected 14 amu shifts in fragments *m/z* 208 and 192. Hence the structure of **31** is as shown here:



The UV spectrum of **31** shows an absorption at 310 nm, which is in good agreement with the chromophore predicted for this structure.[11]

An interesting feature of the ^{1}H and ^{13}C NMR spectra of **31** is that many of the peaks are doubled. Thus, there are two resonances that can be assigned to the H9 amide NH (δ 8.7 and 8.1), whereas that of the H5 amide is a single doublet (δ 9.15, J = 9Hz). Other resonances of the NMR spectra are



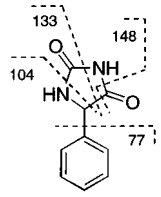
Scheme 1—Proposals for nonoxidative degradation pathways.

presented in Table 3. Such behavior might indicate racemization at one of the asymmetric centers (*e.g.*, C6 and/or C7) of the molecule. This idea could not be confirmed chromatographically, presumably because the retention times of the two isomers were too similar.

Hydrolysis Products—The structures of **14** and **5**, both of which result from hydrolyses of the cefaclor nucleus, were identified by comparison to authentic compounds (see *Experimental Section*).

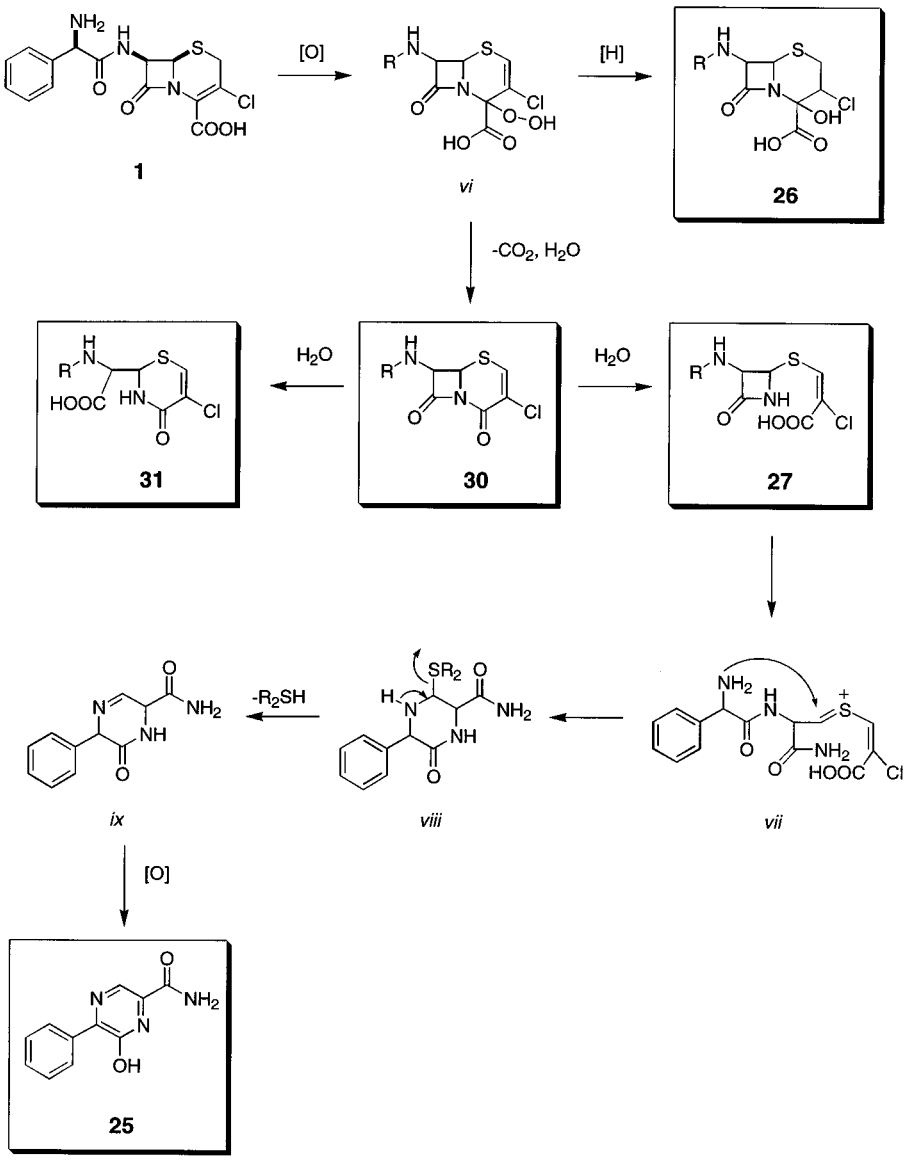
Miscellaneous Structures—Five compounds do not fit into the basic subdivisions of structures already discussed. Three of these structures (**20**, **21**, and **6**) were identified by comparisons with authentic compounds. Spectroscopic data for these compounds are included in Table 4.

*Compound **21***—This product was isolated in small amounts in a fraction contaminated by **24** and **28**. The UV spectrum showed only end absorption and phenyl chromophore. Mass spectrometry indicated that the molecular formula of the major component was $C_9H_8N_2O_2$ (calc for M^+, 176.05858; found, 176.05777), and that the molecule included a phenyl group (*m/z* 77, calc C_6H_5 77.03913, found 77.04099). Losses of CO and CONH also suggested that the structure contained carbonyl and/or amide groupings in a cyclic structure. The fragmentation in the EI-MS and FAB-MS/MS spectra was rationalized in terms of the following proposed structure:



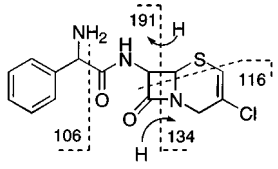
This proposed structure was subsequently confirmed by comparing the NMR and mass spectra to those of an authentic sample of 5-phenylhydantoin.

*Compound **28***—From the molecular formula of **28** ($C_{14}H_{14}N_3O_2SCl$) it can be proposed that this product is formally a decarboxylated cefaclor. The presence of a phenylglycyl moiety is confirmed by the NMR and mass spectra. The IR spectrum shows that the β-lactam has survived (1769 cm^{-1}), a conclusion confirmed by the fragment at *m/z* 191 in the mass spectrum and by the typical pattern of resonances for protons 6, 7, and 9 in the ^1H NMR spectrum. The ^1H NMR spectrum also has peaks assignable to a vinyl proton (δ 6.58) and to an isolated methylene group (δ 4.21 and 3.77). Based on these data, **28** was proposed to be the structure of this compound. This proposal is supported by the detection of fragment peaks at *m/z* 116, 191, and 134 in the MS/MS of the MH^+ peak. The MS studies indicated that fragment ions



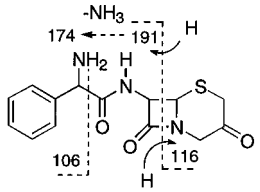
Scheme 2—Proposals for degradation pathways involving C-4 oxidation.

m/z 116 and 134 contained chlorine, and from the accurate mass of the *m/z* 116 fragment it was determined that the elemental composition of this peak is C_4H_3NOCl, which can be rationalized as shown in the following structure:

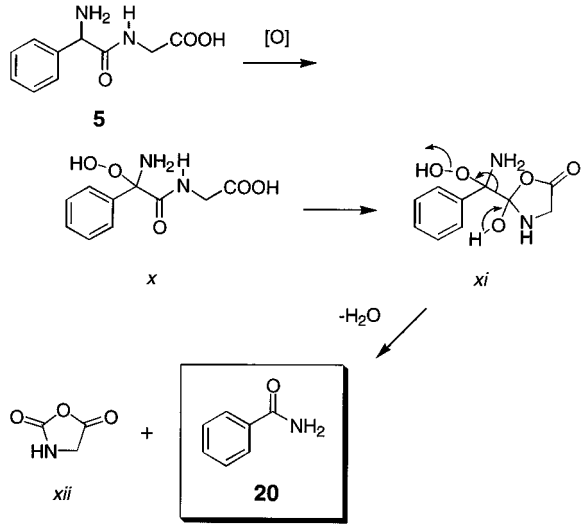


The UV spectrum of **28** is similar to that of **6**. On this basis we propose, but cannot prove, that the double bond is located in the 2−3 position instead of the 3−4 position, as shown in structure **28**.

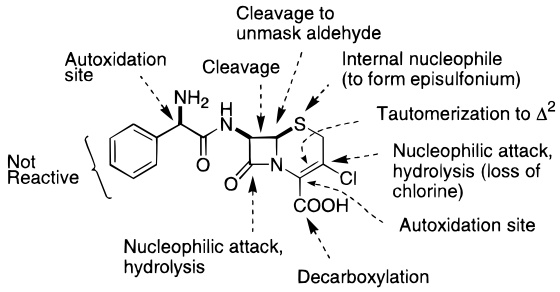
Compound **29**—The molecular formula of **29** ($C_{14}H_{15}N_3O_3S$) indicates that it differs from **28** by the formal replacement of a chlorine atom by a hydroxyl group. A carbonyl stretching frequency in the IR (1765 cm^{-1}) and the usual pattern of peaks in the mass and NMR spectra confirm that the β-lactam has survived. The ^1H NMR spectrum indicates the presence of two isolated methylene groups, and a peak near δ 200 in the ^{13}C NMR spectrum shows that the molecule included a ketone substructure. Therefore **29** is proposed for this product. As shown, the MS/MS of the protonated molecular ion is consistent with this proposal:



Degradation Products of Unknown Structure—As can be seen in Figure 1, four significant peaks present in the HPLC chromatograms of samples of cefaclor aged at room temperature and at 40 °C and 75% RH (chromatograms **B** and **C**, respectively) remain unidentified. These peaks were not present in the 85 °C-degraded cefaclor sample (chromatogram **A**). Attempts to isolate quantities of U_1 for structure elucidation from a sample aged at room temperature showed



Scheme 3—Proposed pathway leading to **20**.



Scheme 4—Overview of chemical degradation of cefaclor.

that the U_1 peak comprises more than one entity, and no further work was done on this fraction. Peaks U_2-U_4 have also not been identified. By comparing the UV spectra of U_1-U_4 with other degradation products, however, we can make some general conclusions. The UV spectrum of U_1 indicates primarily end absorbance, suggesting that the β-lactam moiety is not intact in the degradation products composing this fraction. The UV spectrum of U_2 is similar to thiazole-containing degradation products (*e.g.*, **24**). The UV spectra of U_3 and U_4 are characteristic of substituted 2-hydroxy-3-phenylpyrazine derivatives.

Discussion

Proposed Degradation Pathways—The degradation of cefaclor in solution is initiated by the hydrolysis of the β-lactam ring.[2] In the solid-state degradation of cefaclor,[12] this pathway might be expected to be suppressed. Thus, it is perhaps not surprising that the thiazole and pyrazine products that predominate the degradation of cefaclor in solution are minor products when the solid antibiotic is degraded. With exceptions to be discussed below, we presume that the mechanistic origins of the pyrazines (**3** and **13**), thiazoles (**22**, **23**, and **24**), and simple cleavage products such as **14** and **5** are the same as in solution.

A number of other products, however, are unique to the solid-state degradation. Many of these can be explained by mechanisms that do not require the presence of water, as shown in Scheme 1. For example, Δ^2-cefaclor (**6**) is a major product in all solid-state degradations. A separate experiment showed that **6** was in equilibrium with cefaclor under these conditions. It is reasonable to propose that this tautomeric interconversion is mediated by a protonated carbonium ion such as *i*. This intermediate might decarboxylate to yield **28**, or could be trapped by the small amount of moisture present to form intermediate *iii*, which could serve as a precursor of a second product of decarboxylation, **29**. Whatever the source of **28** and **29**, they are significant products only in the degradation at 85 °C, which suggests that the processes that lead to these products involve energy barriers that preclude their formation at lower temperatures.

At lower temperatures, it appears that C4 oxidation becomes the predominant degradation pathway. As shown in Scheme 2, most of these oxidative products can be proposed to arise from autoxidation of cefaclor to intermediate *vi*. Reduction of *vi* leads directly to **26**. Alternatively, elimination of carbon dioxide and water from *vi* leads to imide **30**. Either of the lactams of **30** could be hydrolyzed to form the ring-opened compounds **31** and **27**. Compounds **26**, **27**, and **31** are important contributors to the product mixtures obtained from degradations **B** and **C**.

It may be worth noting that we have never observed C-4 epimers of **6** and **26**. Of course it is possible that these compounds are among the several minor components that have not yet been characterized, but it appears at present that there is stereoselectivity in the formation of these compounds. At present we have no data permitting speculation regarding the basis of this stereoselectivity, and this remains an interesting subject for future research.

Scheme 2 also suggests a possible mechanism for the formation of the pyrazine **25**. This product cannot be directly explained by the mechanisms proposed for the formation of pyrazines in solution.[2] The attachment of the carboxamide substructure directly to the pyrazine would require the involvement of a compound of the oxidation state of urea in an aldol reaction. In Scheme 2 it is proposed that cleavage of the strained β-lactam leads to a compound that can cyclize to form intermediate *viii*, which by elimination of "RSH" can lead to the formation of an intermediate (*ix*) that could be oxidized to form **25**.

The isolation of benzamide (**20**) provides another mechanistic challenge. As in the case of **25**, the survival of the carboxamide is difficult to explain. One possible mechanistic proposal is shown in Scheme 3. Compound **5**, known to be a product of these degradations, can serve as the starting point of this mechanism. Autooxidation at the benzylic methine leads to a hydroperoxide (*x*), which can be imagined being in equilibrium with a lactol form *xi*. Cleavage with elimination of water leads directly to **20** and the neutral product *xii*.

Concluding Remarks—It has long been known that cefaclor, as well as other cephalosporins, give rise to numerous compounds upon aqueous or solid-state degradation. The structures of these compounds have largely remained undefined because of the complexity of the purification and structure elucidation processes. Our work on the aqueous degradation of cefaclor[2] has delineated the rich chemistry involved in the degradation pathways of cefaclor. In combination with the present report on the solid-state degradation of cefaclor, a comprehensive picture of the reactivity of cefaclor is emerging (Scheme 4). It is now clear that the sulfur atom has a central role in the degradation of cefaclor. This role becomes even more apparent when the degradation of cefaclor is compared with the degradation of the 1-carba analog of cefaclor, loracarbef.[13] The tremendous disparity between the degradation pathways and stability of these two closely related analogs is remarkable. It is hoped that an understanding of the chemistry of the degradation pathways of cefaclor will lead to new insights in understanding the chemical reactivity of other cephalosporin antibiotics.

References and Notes

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10. See Scheme 3 of the accompanying paper (ref 2).
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Acknowledgments

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