Controlled Hydrolysis of Ceftiofur Sodium, a Broad-Spectrum Cephalosporin; Isolation and Identification of Hydrolysis Products

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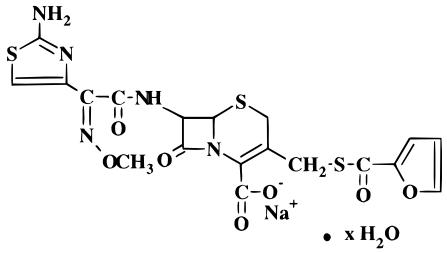
Abstract □ Ceftiofur sodium is the salt of (6*R*,7*R*)-7-{[(2-amino-4-thiazolyl)-*Z*-(methoxyimino)acetyl]amino}-3-{[(2-furanylcarbonyl)thio]methyl}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. This compound is very susceptible to acid, alkaline-, and enzyme-catalyzed hydrolysis, producing a number of unstable degradation products. In this report, we describe the preparation and identification of the hydrolysis products that are formed under controlled alkaline conditions. The primary hydrolysis product was desfuroyl ceftiofur, which is the most abundant metabolite in bovine blood. Desfuroyl ceftiofur was carefully oxidized with H_2O_2 to prepare the disulfide dimer, a urinary metabolite of ceftiofur sodium in the rat and cattle. Under acidic conditions, desfuroyl ceftiofur was converted to the corresponding thiolactone. The preparation of desacetyl cefotaxime, which is the oxygen analog of desfuroyl ceftiofur, is also described. Furoic acid was readily formed by hydrolytic cleavage of the thioester bond. Thiofuroic acid, formed by the less common cleavage on the alkyl side of the thioester bond, was also isolated.

Introduction

Ceftiofur sodium (**I**) is the salt of (6*R*,7*R*)-7{[(2-amino-4-thiazolyl)-*Z*-(methoxyimino)acetyl]amino}-3-{[(2-furanylcarbonyl)thio]methyl}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. Ceftiofur is a third-generation cephalosporin, and its broad spectrum of activity is in part attributed to its resistance to inactivation by bacterial β-lactamase, which is due to presence of a bulky imino methoxy side chain.[1] Human metabolism of various cephalosporins, which differ from ceftiofur only in substitution at the 3 position of the dehydrothiazine ring, has been studied.[2−5] These cephalosporins are primarily excreted unchanged in the urine during the first 24 h. However, cefotaxime (Claforan, Hoechst Roussesl Pharmaceuticals, Inc., Somerville, NJ), which differs from ceftiofur in that the 3-methyl dehydrothiazine ring has an acetate group rather than the thiofuroyate group in ceftiofur, is readily metabolized to desacetyl cefotaxime as the major metabolite in rats, dogs, and humans following intramuscular (im) and intravenous (iv) treatments.[6] The kinetics of the hydrolysis of cefotaxime was studied in detail by Febre *et al.*[7] Desacetyl cefotaxime was identified as the primary hydrolysis product. In acidic medium, desacetyl cefotaxime is converted to the corresponding lactone. Subsequently, the β-lactam ring opens to give unidentified products. In contrast, ceftiofur sodium hydrolysis, under normal conditions, is complex; desfuroyl ceftiofur (**II**) is not seen as a major hydrolysis product because it is readily hydrolyzed or oxidized to more complex products. It was the observation that these side reactions are suppressed in solutions of high ionic strength that helped us to prepare **II** in good yield.



I

Ceftiofur sodium is approved[8] under the trade name Naxcel/Excenel for the treatment of a bovine respiratory infection. The major causative organisms in this complex disease condition are *Haemophilus somnus, Pasteurella haemolytica, and P. multocida*. Ceftiofur sodium is also approved for certain respiratory infections in the swine and day-old chicks.[9,10]

In this report, we summarize the preparation and identification of the major hydrolysis products of ceftiofur sodium formed under controlled alkaline conditions. These isolated compounds were used as reference compounds to aid in the identification of the metabolites of ceftiofur sodium and also in evaluating their respective toxicity.

Experimental Section

Materials—Ceftiofur sodium and ceftiofur hydrochloride were obtained from production lots from The Upjohn Company, Kalamazoo, MI, and were used without any further purification. All other chemicals were laboratory grade. All solvents were distilled in glass quality (Burdick and Jackson, and Muskegon, MI).

Instrumentation—*High-Performance Liquid Chromatography HPLC Methodology*—Two HPLC systems were used to monitor and quantitate ceftiofur sodium and its degradation products; these were isocratic ion-pair system and gradient HPLC. The isocratic ion-pair system was the standard method for the analysis of ceftiofur sodium and for monitoring the presence of hydrolysis products. The isocratic ion-pair system consisted of any standard HPLC instrument and a 254 nm UV detector. The column was 25 × 0.46-cm 5-μm Zorbax C_8 (DuPont) or equivalent. The aqueous portion of the mobile phase was 0.05 M ammonium acetate containing 1.95% (v/v) of a 40% (v/v) solution of tetrabutylammonium hydroxide. The pH of this solution was adjusted to 6.6−6.8 with glacial acetic acid. The total mobile phase consisted of 70% (v/v) aqueous phase, 20% (v/v) methanol, and 10% (v/v) tetrahydrofuran. Acetophenone was used as a convenient internal reference standard when needed because it was well resolved from ceftiofur sodium and the major degradation products. At a flow rate of 1 mL/min, the retention times were ~12 and 14 min for ceftiofur sodium and acetophenone, respectively.

Gradient HPLC was used extensively in this study to achieve adequate separation of the known and unknown degradation products of ceftiofur sodium. This method was developed by Krzeminski *et al.*[11] A Varian 5500 or equivalent HPLC equipped with a 254 nm UV detector was used. The column was 100 × 4.6 mm containing Bakerbond wide pore octyl (C_8), 300 Å pore size, 5-μm spherical packing material. A 60-min gradient was used starting with 100% water containing 0.1% (v/v) triflouracetic acid (TFA) for (5 min) to 16% (v/v) acetonitrile containing 0.1% (v/v) TFA for 13 min, and then

‡ Retired from The Upjohn Company.

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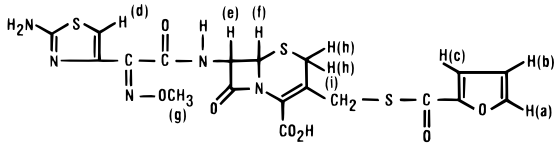
Table 1—Proton NMR Chemical Shift Assignment for Ceftiofur Sodium and the Major Hydrolysis Products



CEFTIOFUR (FREE ACID)

assignments	Chemical Shifts, ppm			
	Ceftiofur	II	IV	V
a (Aromatic doublet)	7.74	Absent	Absent	Absent
b (Aromatic doublet)	6.62	Absent	Absent	Absent
c (Aromatic doublet)	7.33	Absent	Absent	Absent
d (Aromatic singlet)	6.95	7.06	6.8	7.00
e (CHN)	5.78	5.80	5.94	5.80
f (CHN)	5.17	5.26	5.16	5.20
g (OCH_3)	3.97	4.03	3.96	4.03
h (1/2 CH_2AB)	3.33	3.35	3.93[a]	3.53
h (1/2 CH_2AB)	3.64	3.56	3.87[a]	3.80
i (1/2 CH_2AB)	3.91	3.57	3.74[a]	3.70
i (1/2 CH_2AB)	4.20	3.74	3.33[a]	4.12
Solvent	D_2O	D_2O	CD_3OD	CD_3OD

[a] These are unassigned peaks.

to 19% (v/v) organic phase for 43 min. The column was then flushed with 90% (v/v) organic phase.

Structural Analysis—Proton nuclear magnetic resonance (NMR) data was aquired on a Varian XL 300 FT NMR with solvents as listed in Table 1. The infrared (IR) spectra were recorded on a Digilab model FTS15E spectrophotometer after making KBR pellets of the respective compound. Thermospray mass spectra were obtained on a Finnigan 4600 quadrupole mass spectrometer equiped with a Finnigan thermospray interface using a 30:70 methanol:0.05 ammonium acetate mobile phase. Fast-atom bombardment (FAB) mass spectra were obtained on a Finnigan MAT CH5-DF instrument with a xenon atom beam.

Preparation of Desfuroyl Ceftiofur—*Hydrolysis in Saturated Potassium Chloride Solution*—Fifty milliliters of saturated KCl containing 0.5 g of tetrasodium EDTA and 0.4 g sodium bisulfite was deaerated by sonication and cooled to 4 °C. One gram of ceftiofur sodium was added and dispersed by sonication. The mixture was again cooled to near 0 °C and stirred with a magnetic stirring bar under a N_2 atmosphere. To this solution, 3 mL of cold deaerated 22.5% (w/v) KOH solution containing 0.5% (w/v) tetrasodium EDTA was added in a dropwise manner. After a 1-h incubation under N_2 at 0 °C, all of the ceftiofur sodium was hydrolyzed, as determined by HPLC analysis. The pH of the hydrolyzate was then adjusted to 2.5 with cold 20% (v/v) H_3PO_4. An off-white precipitate was obtained. The suspension was cooled in an acetone-dry ice bath to coagulate the precipitate and then centrifuged in 50-mL tubes, and the supernatant was discarded. The precipitate was washed twice with cold deaerated 0.2% (v/v) acetic acid and once with cold deaerated water. The cake was suspended in ~30−40 mL of cold deaerated water and lyophilized. About 0.4 g of a light yellow powder was obtained. This material was purified on a 2-g C_{18} silica column eluted with cold deaerated water. The effluent, collected in 2-mL fractions, was monitored by HPLC for purity. The fractions containing the least amount of impurities were combined and lyophilized. The product was a white amorphous powder with HPLC purity in the 75−85% range.

Hydrolysis of a Suspension in Methylene Chloride—About 1 g of ceftiofur sodium or ceftiofur hydrochloride was suspended in 90 mL of methylene chloride. Thirty milliliters of 1 N KOH saturated with KCl and also containing 0.5% (w/v) disodium EDTA was added. The suspension was gently mixed and left at room temperature under a blanket of N_2 for 0.5 to 1 h. When the reaction was complete, as monitored by HPLC, the aqueous phase was separated and adjusted to pH 2.5−3.0 with 20% (v/v) H_3PO_4. The suspension was cooled in an acetone-dry ice bath to coagulate the precipitate. The precipitate was washed with 0.2% (v/v) acetic acid and water and lyophilized as already described. About 0.4 g of a light yellow powder was obtained. An aqueous solution of this material was purified as already described on a 2-g C_{18} silica column. The final product was a very white amorphous powder with HPLC purity in the 75−85% range.

Reductive Hydrolysis with Dithioerythritol—One gram of ceftiofur sodium was dissolved in 20 mL of water in a 50-mL glass stoppered centrifuge tube. Ten milliliters of an aqueous solution of 4% each of dithioerythritol and triethylamine was added. The mixture became cloudy and thick. The tube was immersed in a water bath at 45−50 °C and heated until clear (15−20 min). The pH of the clear solution was adjusted to 2.5−3.0 with cold 20% (v/v) H_3PO_4 and cooled at −10 °C. The precipitate was washed free of furoic acid, dithioerythritol, and inorganic salts with cold 0.2% (v/v) acetic acid. It was then lyophilized from water to obtain an amorphous white powder.

Preparation of Desfuroyl Ceftiofur Dimer: Hydrolysis of Ceftiofur Sodium Followed by Oxidation with H_2O_2—Ceftiofur sodium was hydrolyzed to desfuroyl ceftiofur according to the procedure just described. The pH of the hydrolyzate was adjusted to 6 with 20% (v/v) H_3PO_4 while stirring under a blanket of N_2. Four milliliters of cold 10% (v/v) H_2O_2 was added and allowed to stand for ~1 h or until the dimerization was complete. If the solution was too thick, it was diluted with water. The pH was then adjusted to 2.5−3.0 with cold 20% (v/v) H_3PO_4. The precipitate was separated by centrifugation. The furoic acid and inorganic salts were removed by two to three washings with cold 0.2% (v/v) acetic acid followed by centrifugation. The precipitate was then suspended in water and lyophilized. The final product was a white amorphous powder that produced a single peak when analyzed by gradient elution HPLC, with an area percentage purity of over 90%.

Preparation of Desfuroyl Thiolactone—About 1 g of ceftiofur sodium was hydrolyzed to desfuroyl ceftiofur according to any of the procedures already described. To an aqueous suspension of the hydrolyzate, 45 mL of cold 4.5 N methanolic HCl was added in small portions with mixing. This mixture was allowed to stand overnight under a blanket of N_2. Seventy-five milliliters of water was added and extracted with 2 × 50 mL of ethyl acetate and 1 × 50 mL of CH_2Cl_2. The organic washes were discarded. The aqueous phase was transferred to a beaker and the pH was adjusted to 4.2 with solid $NaHCO_3$. The white precipitate was cooled, filtered, and washed with a small quantity of ice cold water and dried under reduced pressure. About 0.3 g of an off-white powder was obtained, and gradient elution HPLC showed it to be essentially one component.

Preparation of the Oxygen Analog of Desfuroyl Ceftiofur (Desacetyl Cefotaxime)—This compound was conveniently prepared by base hydrolysis of cefotaxime. To 0.5 g of cefotaxime suspended in 80 mL of CH_2Cl_2, 30 mL of 0.1 N KOH containing 0.05% tetrasodium EDTA was added, mixed gently, and allowed to stand until the hydrolysis was complete (~1 h). The aqueous phase was separated, and the pH was adjusted to 5.6 with 1 N HCl and with 0.1 N HCl as the pH approached 5.6. The desacetyl cefotaxime that was in solution at this pH was lyophilized to yield a white powder. An aqueous solution of this material was charged onto a 2-g C_{18} silica column and eluted with cold deaerated water. The eluant was collected in 2-mL fractions that were monitored by HPLC for purity. The fractions containing most of the compound were combined and lyophilized. The product was a white amorphous powder.

Preparation of Thiofuroic Acid as the Thioester—One hundred milligrams of ceftiofur sodium was dissolved in 2 mL of 0.01 M $NaHCO_3$ in a stoppered test tube and heated on a steam bath for 2 h. The generated thiofuroic acid was derivatized to its methylester by adding 0.3 mL of methyl iodide in 1 mL of ethyl acetate and allowing to stand overnight. The lower phase, which appeared to be a semisolid, was separated by decantation and partitioned with 3 mL hexane. The hexane phase was evaporated under N_2. HPLC and TLC of the residue showed a single component.

Results and Discussion

Ceftiofur sodium undergoes extensive degradation in alkaline and acid solutions, producing a large number of compounds as seen by HPLC with UV detection. Figures 1A and 1B are chromatograms of alkaline- and acid-degraded solutions of ceftiofur sodium, respectively.

Metabolic studies of ceftiofur sodium in cattle indicate that it is immediately metabolized to a more polar compound,

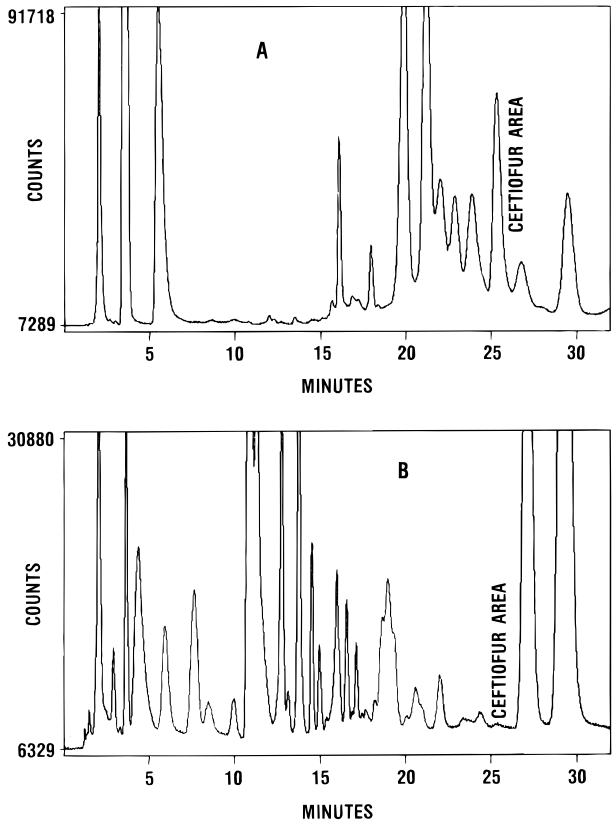
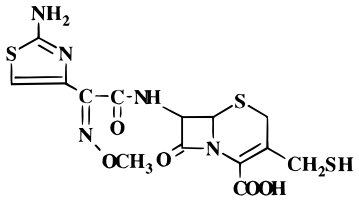


Figure 1—Gradient elution HPLC chromatograms of degraded ceftiofur sodium solutions: (A) 0.01 N NaOH; (B) 0.1 N methanolic HCl.

which has been identified as desfuroyl ceftiofur (**II**).[1] It is now believed that the *in vivo* efficacy of ceftiofur sodium is mainly due to desfuroyl ceftiofur and related metabolites. The disulfide dimer of desfuroyl ceftiofur (**V**) has been identified in the urine of ceftiofur-treated rat and cattle.[1] The thiolactone of desfuroyl ceftiofur (**IV**) was detected in the rat and cattle.[1] It was necessary to prepare all of these compounds in as pure a form as possible and in adequate quantity for investigational purposes. As pointed out earlier, simple hydrolytic conditions were not useful. We achieved our goal by using empirically determined reaction conditions.

Desfuroyl ceftiofur (**II**) was expected to be the primary hydrolysis product of ceftiofur sodium because its formation involved the cleavage of the labile thioester bond. We found



II

that an effective way of suppressing the side reactions, resulting in the generation of a number of compounds, was to increase the ionic strength of the hydrolysis media with a saturated KCl solution (see *Experimental Section*; Figure 2). The first peak in Figure 2 is furoic acid and the second peak is desfuroyl ceftiofur. After precipitation, the entrained free furoic acid was removed by washing with 0.2% (v/v) acetic acid. It was important that the suspension of the precipitated desfuroyl ceftiofur was frozen and lyophilized immediately to avoid decomposition.

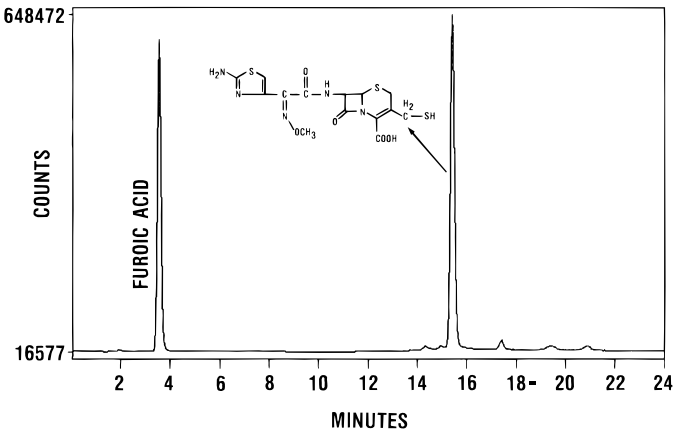


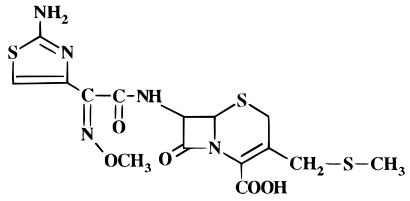
Figure 2—Gradiet elution HPLC chromatogram after controlled hydrolysis of ceftiofur sodium: generation of furoic acid and desfuroyl ceftiofur.

The second procedure described in the *Experimental Section* is essentially the same as the first except it involved the transfer of a suspension of ceftiofur sodium in methylene chloride into 1 N KOH solution saturated with KCl.

The third procedure detailed in the *Experimental Section* involved the use of the disulfide reducing agent dithioerythritol which is widely used in the reduction of disulfide bonds in polypeptides and proteins.[12]

The structure of **II** was confirmed by IR, proton NMR, and thermospray mass spectral data, which are shown in Figures 3, 4, and 5A, respectively. In addition to a strong pseudomolecular ion at 430 Da, the spectrum of **II** also shows ions at *m/z* 452 and 468 Da, representing the sodium and potassium adducts of **II**, respectively. The IR spectrum supports the structure except for the uncertainty of the free sulfhydryl group. The proton NMR spectrum may be interpreted by comparing the chemical shifts of ceftiofur sodium with that of **II**, as shown in Table 1. The chemical shifts are in good agreement for the proposed structure, but the NMR spectra cannot confirm or deny the presence of the sulfhydryl group.

The presence of the sulfhydryl group was confirmed by preparing the methylmercaptide (**III**) by reacting **II** with methyl iodide. The mass spectra showed an ion at *m/z* 444,



III

representing M + H^+ and other ions consistent with the proposed structure.

Compound **II** has a relative retention time (all relative retention times are with reference to that of ceftiofur sodium) of ≈0.57 on the ion-pair system and ≈0.55 on the gradient HPLC system (see *Experimental Section*).

Compound II was isolated as a white amorphous powder that is fairly stable in the dry state, decomposes quickly in alkaline solution, is stable in a mild acidic solution, and it is converted into the thio-lactone **IV** in a strongly acidic medium.

Compound **IV** is formed in ceftiofur-treated cow plasma and urine that was acidified with trifluoroacetic acid to prevent the metabolite **II** from decomposing.[1] The FAB mass spectrum of **IV** (Figure 5C) shows a strong [M + H^+] ion at 412 Da. The proton NMR spectrum is shown in Figure 4. The

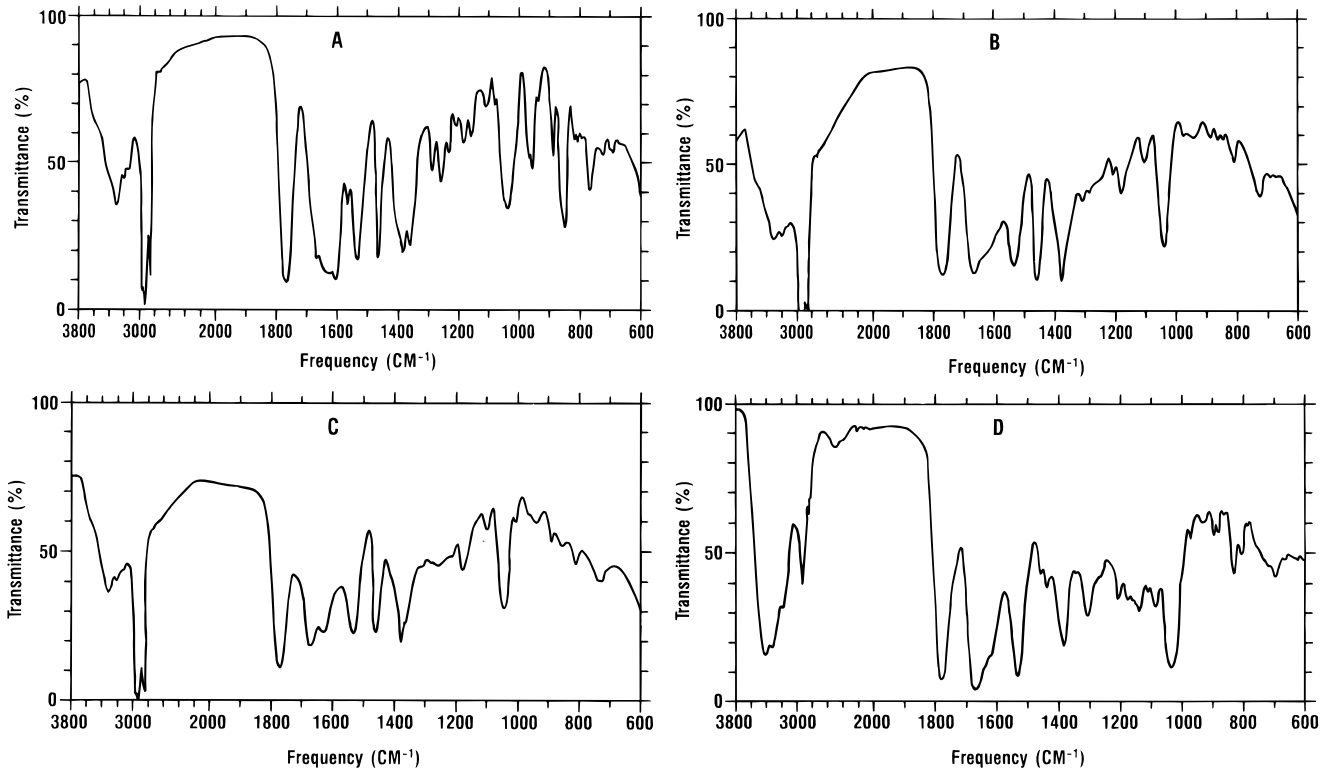
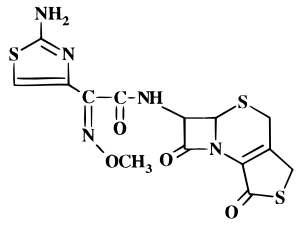


Figure 3—The IR spectra of ceftiofur and hydrolysis products: (A) ceftiofur sodium, mineral oil mull; (B) desfuroyl ceftiofur, mineral oil mull; (C) desfuroyl ceftiofur dimer, mineral oil mull; (D) desfuroyl ceftiofur thiolactone, micro KBR.

chemical shifts for **IV** are shown in Table 1 along with those for **I**. The data indicate an absence of furoic acid and the presence of shifts at 6.8, 5.94, 5.16, and 3.96 ppm assigned, respectively, to the d, e, f, and g protons in the structure shown in Table 1. The spectrum also shows shifts at 3.93, 3.87, 3.74, and 3.33 ppm, which are due to the protons at the h and i positions. However, there is no AB pattern seen for these two CH_2 groups.

The IR spectrum (Figure 3) may be compared with the spectra of **I** and **II** (Figure 3A and 3B). The spectrum shows all the characteristic bands and the absence of 1700 and 1612 cm^{-1} bands (COOH and COO^-). From the spectral data and the reported chemical behavior of the oxygen analog of **IV** generated from the commercially available cephalosporin cefotaxime,[7] **IV** is assigned the following structure: Com-

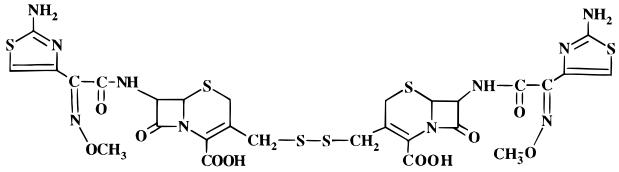


IV

pound **IV** has a relative retention time of ≈0.52 min in the gradient HPLC system.

In the alkaline hydrolysis of **I**, one of the degradation products has a relative retention time of 0.93 min in the gradient HPLC system. Krzeminski *et al.*,[11] in their study on the *in vitro* metabolism of **I** in heifer plasma, found a metabolite with the same relative retention time. This compound was identified as the dimer of **II**.

This dimer was prepared in a pure form and high yield by the selective hydrolysis of **I** to **II** and subsequent oxidation of **II** with H_2O_2 to **V** (see *Experimental Section*). Compound **V** was assigned the following structure based on its thermospray mass spectrum (Figure 5B) and its IR and proton NMR spectra (Figures 3 and 4). The IR spectrum shows the



V

presence of the β-lactam carbonyl at 1773 cm^{-1}, indicating that the β-lactam is intact. There is no indication of the furan thioester absorbance near 960 cm^{-1}.

The proton NMR spectrum showed the expected AB pattern of the two methylene protons adjacent to the sulfur in the cephalosporin ring system and the two methylene protons on the 3 position adjacent to the disulfide sulfur. The spectrum showed the expected pattern for the two adjacent protons in the β-lactam ring. The singlet for the methoxy group of the methoxine and the proton on the aminothiazole ring were present. No furan protons were indicated. In general, the chemical shifts and patterns were like ceftiofur except for no furan protons.

The mass spectrum had an ion at 857 Da, which agrees with the expected disulfide [M + H^+]. The ion at 429 represents cleavage at the disulfide bond and the ion at 243 comes from the cleavage of the aminothiazole side chain from the cephalosporin ring. These spectra all support the assigned disulfide structure.

Furoic acid (**VI**) is generated from ceftiofur sodium under normal hydrolytic conditions. It has a relative retention time of ≈0.29 min in the ion-pair HPLC system. When ceftiofur

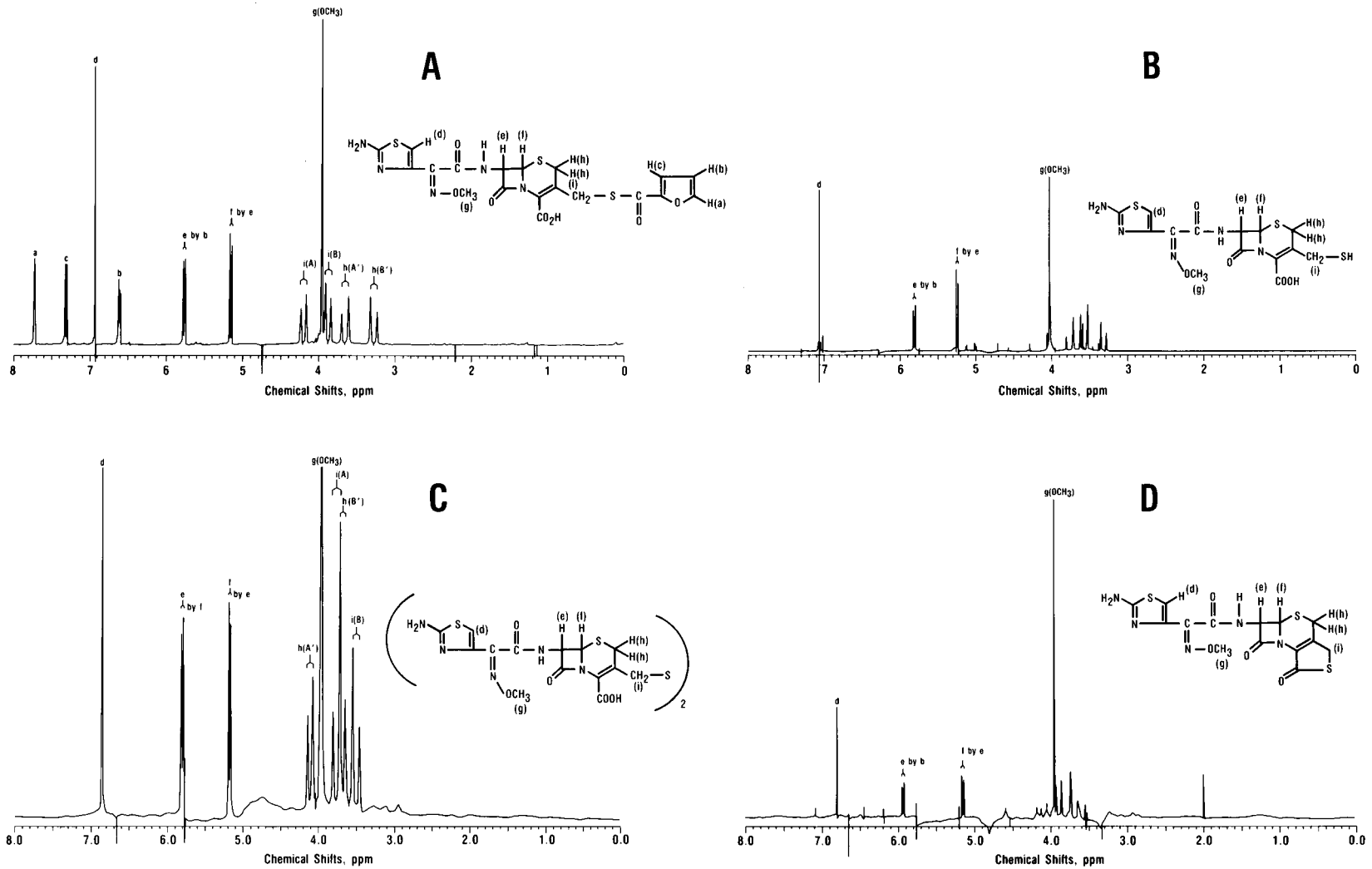


Figure 4—Proton NMR spectra of ceftiofur and hydrolysis products (see Table 1 for chemical shift assignments): (A) ceftiofur, free acid; (B) desfuroyl ceftiofur; (C) desfuroyl ceftiofur dimer, (D) desfuroyl ceftiofur thiolactone.

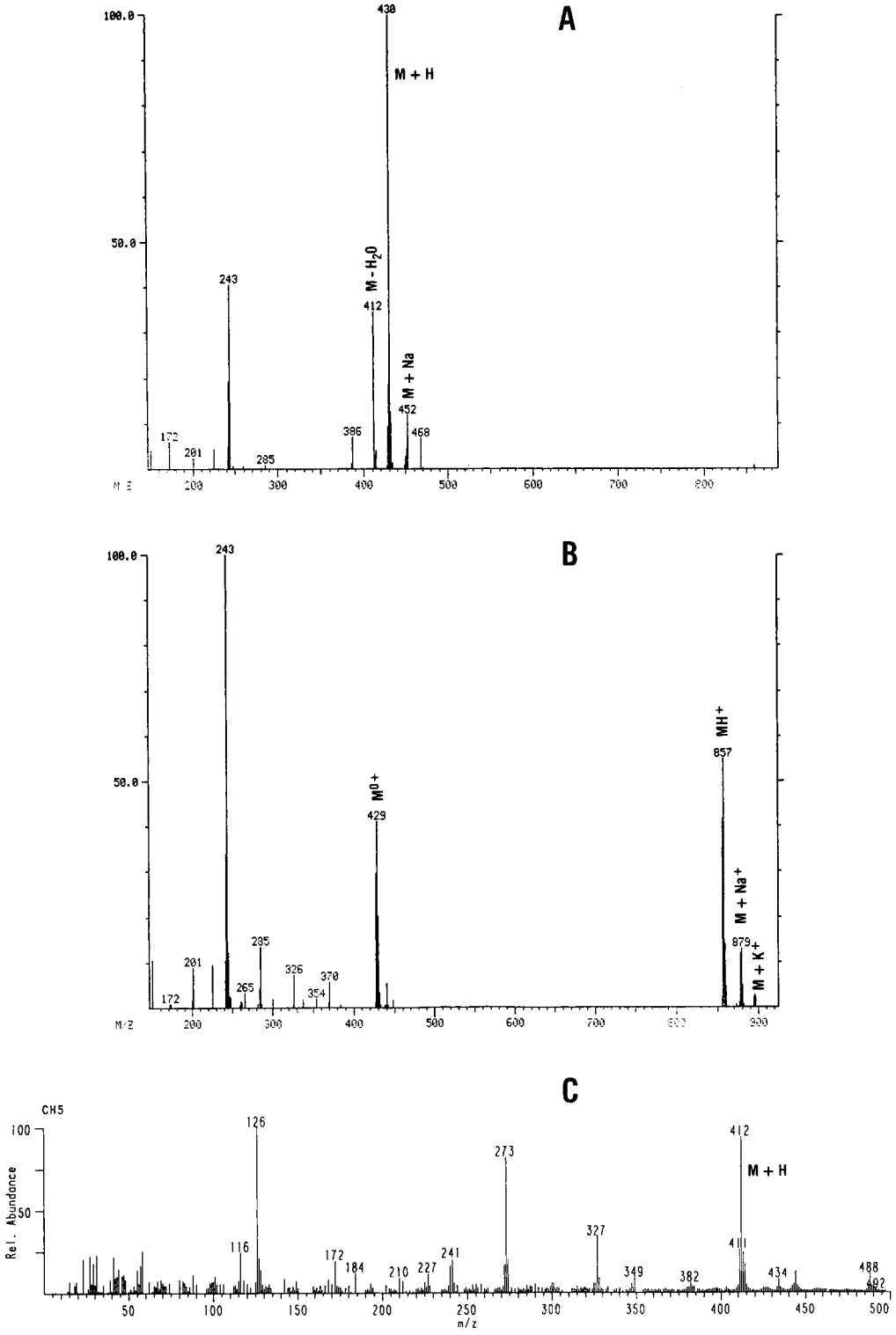


Figure 5—Mass spectra (thermospray) of ceftiofur hydrolysis products: (A) desfuroyl ceftiofur; (B) desfuroyl ceftiofur dimer; (C) desfuroyl ceftiofur thiolactone (FAB-MS).

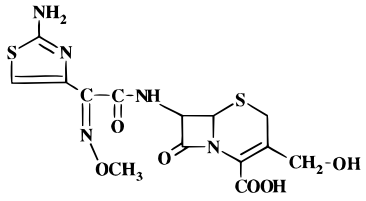
was hydrolyzed in 0.01 M $NaHCO_3$ (see *Experimental Section*), an additional peak was observed with a relative retention time of 0.42 min. Subsequent derivatization with methyl iodide revealed this material as the methyl ester of thiofuroic acid by comparison of retention times on the ion-pair HPLC and by gas chromatography/mass spectroscopy (GC/MS) of an authentic standard.

The formation of thiofuroic acid (**VI**) under rather mild hydrolytic conditions is interesting because it involves an

VI

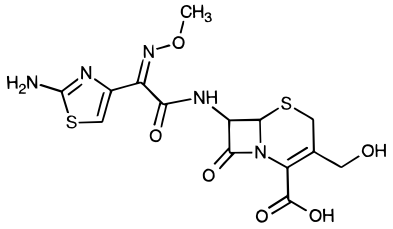
uncommon mechanism in the cleavage of an ester bond. There are two pathways by which nonenzymatic hydrolysis of *N*-acylated 7-aminocephalaoporanic ester type of cephalosporins (cephalothin, cephaloglycin, and cefotaxime) may

proceed. The more common path involves acyl-oxygen bond cleavage, but alkyl-oxygen bond cleavage may occur if the intermediate carbonium ion is particularly stable.[13] Indelicato *et al.*[14] studied the hydrolysis of cephalothin (a C-3′-acetoxy *N*-acylated 7-aminocephalosporanic acid cephalosporin) with the use of isotopically labeled $H_2{}^{18}O$ and [2-^{13}C] acetate ion. The ^{18}O incorporation studies showed that the hydrolysis to the desacetyl derivative of cephalothin proceeds via two distinct pathways: 55−63% cleavage of the alkyl-oxygen bond and the remainder by the acyl-oxygen bond cleavage. In ceftiofur hydrolysis, acyl-sulfur bond cleavage was the more predominant cleavage under normal conditions. In 0.01 M $NaHCO_3$, it appeared that the alkyl-sulfur bond cleavage was very much evident, though in low yield.



VII

Desacetyl cefotaxime **VIII** is the oxygen analog of desfuroyl ceftiofur (**II**). It was impractical to prepare **VIII** by controlled oxidation of **II**. Therefore, we prepared **VIII** by hydrolyzing the commercially available antibiotic cefotaxime. The hydrolysis was carried out in a manner similar to the preparation of **II** from **I** as described in the *Experimental Section*. The structure of II was confirmed by IR, NMR, and MS techniques.



VIII

Summary

Human metabolism of various cephalosporins, which differ from ceftiofur only in substitution at the 3 position of dehydrothiazine ring, has been studied recently.[2−5] These cephalosporins are primarily excreted unchanged in the urine during the first 24 h. However, cefotaxime, which has a labile side chain, is readily metabolized to desacetyl cefotaxime as the major metabolite in rats, dogs, and humans following im and iv treatments.[6] The kinetics of the hydrolysis of cefotaxime was studied in detail by Febre *et al.*[7] Desacetyl cefotaxime was identified as the primary hydrolysis product. In acidic medium, desacetyl cefotaxime is converted to the lactone. Subsequently, the β-lactam ring opens to give unidentified products. In contrast, ceftiofur sodium hydrolysis is complex; desfuroyl ceftiofur is not seen as a major product because it is readily hydrolyzed or oxidized to more complex products. It was the observation that these side reactions are suppressed in solutions of high ionic strength that helped us to prepare **II** in sufficient quantity and purity for investigational purposes. We have proven the structure of **II** from its IR, NMR, and MS data. Compound **II** was readily oxidized to prepare its dimer, **V**. The thiolactone of **II** (compound **IV**) was prepared by acidic dehydration of II. Generation of thiofuroic acid from ceftiofur sodium in 0.01 M $NaHCO_3$ is interesting because it involves cleavage of the alkyl bond of the thiol ester linkage. The preparation of the various hydrolysis products by rather unconventional methods was very useful from a practical standpoint. These compounds were used as reference materials in the isolation and identification of the metabolites of ceftiofur in cattle and the rat by Jaglan *et al.*[1] Availability of sufficient amounts of desfuroyl ceftiofur and the disulfide dimer were also useful in evaluating the pathology and toxicology of ceftiofur sodium.

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