as well as nonpolar systems. An effort is being made to obtain W without solubility data, with the possibility of finding a physicochemical basis for W and/or a group contribution method for estimating this solvent–solute interaction term.

It is interesting to observe in Table III and in Figs. 1 and 5 that small (fractional to 1 or 2%) differences between $\delta_1\delta_2$, the geometric mean, and W, the correct adhesive energy density, may cause large differences (25–75%) between ideal and real solubilities. It will challenge the investigator to measure and calculate energies within 5–50 cal/mole required for an independent measure of W for accurate estimation of solubilities. The prediction of solubility using W from group contributions would represent a step toward a better understanding of the behavior of drug molecules in polar and nonpolar solvent systems.

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ACKNOWLEDGMENTS

Supported in part by an endowed professorship to A. Martin by Mr. Coulter Sublett.

The authors acknowledge the assistance of P. L. Wu, who obtained the regression equation of δ_1 *versus* dielectric constant and T. Velasquez for solubility determinations of theophylline in mixtures of polyethylene glycol and water. They also thank Terri Shrode and Nancy Irvin for secretarial assistance.

Degradation Kinetics and Mechanism of Aminocephalosporins in Aqueous Solution: Cefadroxil

AKIRA TSUJI *x, EMI NAKASHIMA *, YOSHIHARU DEGUCHI *, KAZUNORI NISHIDE *, TAKAYOSHI SHIMIZU §, SUMIO HORIUCHI §, KIYOYASU ISHIKAWA §, and TSUKINAKA YAMANA ‡

Received December 12, 1980, from the **Faculty of Pharmaceutical Sciences and the* ‡*Hospital Pharmacy, Kanazawa University, Takaramachi, Kanazawa 920, Japan,* and the §*Nonclinical Research Laboratories, Bristol Banyu Manufacturing Co., Ltd., Kota-cho, Nukata-Gun, Aichi 444-01, Japan.* Accepted for publication March 3, 1981.

Abstract □ The degradation kinetics and mechanism of a new, orally effective cephalosporin derivative, cefadroxil, in aqueous solution were investigated at pH 2.51–11.5 at 35° and ionic strength 0.5. The degradation rates were determined by high-pressure liquid chromatography. At constant pH and temperature, the degradation followed first-order kinetics and a log k–pH profile was presented. The shape of the rate–pH profile resembled that for cephalexin or cephradine under the same conditions. Citrate and phosphate buffers enhanced general acid and base catalysis of the degradation. In aqueous solution, cefadroxil was shown to degrade by three parallel reactions: (*a*) intramolecular aminolysis by the C-7 side-chain amino group on the β-lactam moiety, (*b*) water-catalyzed or spontaneous hydrolysis, and (*c*) β-lactam cleavage by the nucleophilic attack of hydroxide ion. In neutral and weak alkaline solutions, the main degradation products were two piperazine-2,5-diones and 3-hydroxy-4-methyl-2(5*H*)-thiophenone, the former being formed from Reaction *a*, while the latter arose *via* the degradation pathways of Reactions *b* and/or *c*.

Keyphrases □ Cefadroxil—degradation kinetics and mechanism, high-pressure liquid chromatography, pH–rate profile, intramolecular aminolysis to produce piperazinediones, buffer and temperature effects □ Degradation kinetics—cefadroxil, high-pressure liquid chromatography assay, pH–rate profile, intramolecular aminolysis to produce piperazinediones, buffer and temperature effects □ pH–rate profile—cefadroxil, degradation kinetics and mechanism, intramolecular aminolysis to produce piperazinediones, buffer and temperature effects □ Piperazinediones—cefadroxil degradation kinetics and mechanism, intramolecular aminolysis

A previous study (1) determined the degradation kinetics of a series of cephalosporins in aqueous solution at 35° and ionic strength 0.5. The degradation of cephalosporins possessing an α-amino group in their C-7 side chain, such as cephalexin, cephradine, and cephaloglycin, was facilitated by the intramolecular attack of the amino group to the reactive β-lactam moiety at neutral pH (1–4). The relative instability of cephaloglycin under physio-

0022-3549/81/1000-1120$01.00/0

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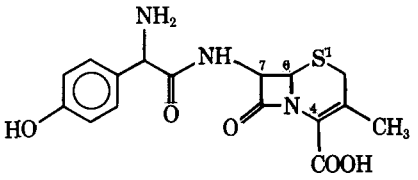
logical conditions was attributed to this phenomenon, its half-life being only ~5 hr. Although cephalexin and cephradine react similarly, they were much more stable than cephaloglycin under the same conditions. Because of the intramolecular aminolysis of these aminocephalosporins in aqueous solution, stable piperazinediones were formed (1–3, 5–7).

This paper describes the degradation kinetics and mechanisms of a new, orally effective cephalosporin antibiotic, cefadroxil (I), which shows essentially the same antibacterial spectrum as cephalexin (8). It is structurally similar to cephalexin, differing only in the hydroxylation of the phenyl side chain.



I

EXPERIMENTAL[1]

Materials—Cefadroxil monohydrate (947 μg/mg) was used without further purification. Buffers and all other chemicals were reagent grade.

Buffers—Citrate and phosphate buffers (pH 2.51–5.82 and 6.00–7.20, respectively) were used for the kinetic study. At pH > 7.20, a pH-stat[2] was used to maintain a constant pH. All buffers of the kinetic solutions were adjusted with potassium chloride to an ionic strength of 0.5. The pH was measured[2] at the temperature of the kinetic study.

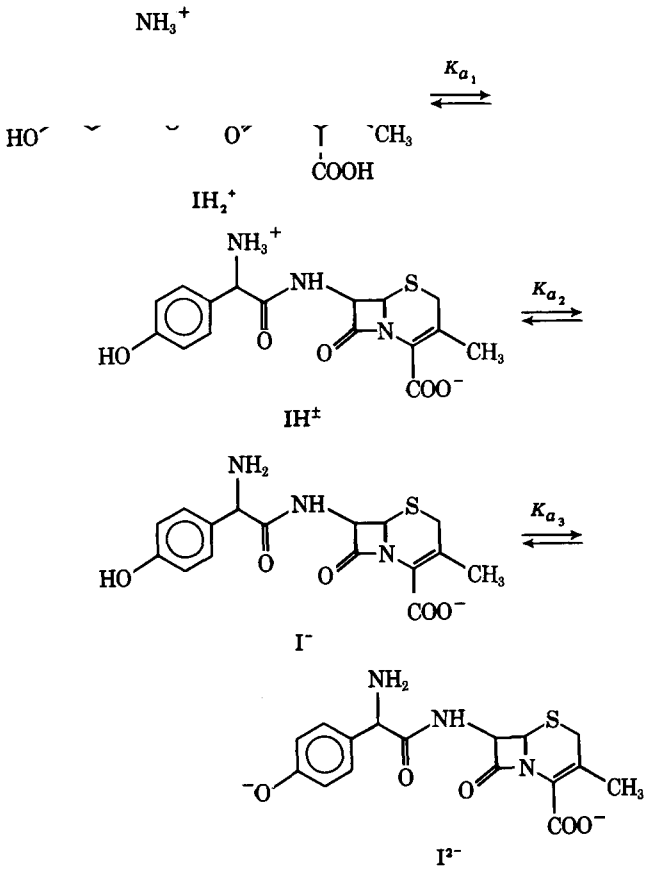
Kinetic Procedures—All kinetic experiments were carried out at 35 ± 0.1° and ionic strength 0.5 unless otherwise stated. Accurately weighed cefadroxil was dissolved in an appropriate buffer or 0.5 *M* potassium chloride solution preheated to the desired temperature to give an initial concentration of 5×10^{-3} *M*. The fast reaction was performed in a glass-stoppered 100-ml volumetric flask. Where the half-life was >1 day, the degradation buffer solution was sealed in 5-ml ampuls. Samples were withdrawn at suitable intervals, cooled in an ice bath, diluted with distilled water if necessary, and analyzed.

The degradation rate of cefadroxil was followed by measuring the remaining drug concentration by a reversed-phase high-pressure liquid chromatographic (HPLC) method. In addition to the HPLC determination of the overall degradation rate of cefadroxil, the decrease in the concentration of the primary amino group in cefadroxil during the degradation was followed by a colorimetric assay.

Analytical Procedures—*HPLC*—The liquid chromatograph[3] was equipped with a UV detector[4] set at 254 nm and a 4.0 × 300-mm stainless steel column prepacked with octadecylsilane chemically bonded on totally porous silica gel[5]. The mobile phase was 2% (or 3%) acetonitrile–98% (or 97%) 0.01 *M* ammonium acetate aqueous solution. Chromatography was performed at ambient temperatures, and samples were eluted at a flow rate of 2 ml/min. Peak heights were measured, and the concentrations were calculated from the calibration curves obtained daily.

Colorimetric Assay—The primary amino group was quantitated by using the trinitrobenzenesulfonic acid assay of Satake *et al.* (9) in a modified form used previously (3) for cephalexin degradation.

A 500-μl aliquot of the degradation solution containing cefadroxil was added to 2 ml of a 0.2 *M* phosphate buffer at pH 7.6. Thereafter, 2 ml of a 0.2% (w/v) aqueous solution of 2,4,6-trinitrobenezenesulfonic acid was added; after standing for 30 min in darkness at room temperature, the absorbance of the resulting orange solution was measured[6] at 420 nm against a blank consisting of 500 μl of reaction solution without cefadroxil, 2 ml of phosphate buffer, and 2 ml of trinitrobenzenesulfonic acid solution treated similarly.



Scheme I

Determination of Ionization Constants—The apparent ionization constants, pK_{a_1} and pK_{a_2}, for the dissociation of carboxylic acid and the α-ammonium group of cefadroxil (Scheme I) were determined to be 2.64 ± 0.03 and 7.30 ± 0.03, respectively, by the potentiometric titration of 0.01 *M* antibiotic aqueous solution at an ionic strength of 0.5 and 35°. The pK_{a_3} value for the phenolic group of cefadroxil was determined spectrophotometrically (10). The calculation was made from the change in absorbance[6] at 250 nm for 5×10^{-5} *M* cefadroxil solution adjusted to ionic strength 0.5 with potassium chloride at various pH values and 35° to give $pK_{a_3} = 9.69 \pm 0.06$.

TLC—TLC was conducted on precoated silica gel plates 0.25-mm thick. Developing solvents were: Solvent A, chloroform–ethyl acetate–acetic acid–water (4:4:4:1); Solvent B, methyl isobutyl ketone–acetic acid–water (7:2:1); Solvent C, toluene–butanol–acetic acid–water (4:4:3:1); and Solvent D, ethyl acetate–isopropanol–water (10:7:5). The chromatograms were visualized by exposing the plates to iodine vapor.

Isolation and Identification of Degradation Products in Basic Aqueous Solution—Cefadroxil (2.5 g) in 0.1 *M* disodium phosphate solution (50 ml) was kept at 60° for 1 hr at pH 9.1. The reaction mixture gave four spots on TLC. Acidification with dilute hydrochloric acid and subsequent fractionation as shown in Scheme II gave the product II (25 mg) and a mixture of IV and V slightly contaminated with III (370 mg).

Product II—Recrystallization from chloroform–hexane yielded the pure compound, mp 118–119.5°; IR: 3370 (OH), 1690 (thiolactone), and 1630 (olefin) cm^{-1}; PMR (deuterochloroform): δ 2.09 (s, 3H, CH_3), 3.74 (s, 2H, CH_2), and 6.10 (s, 1H, OH) ppm; UV (water-methanol, 9:1): λ_{max} 246, 255 (shoulder), 246, and 304 nm (0.1 *N* NaOH added).

Anal.—Calc. for $C_5H_6O_2S$: C, 46.14; H, 4.65. Found: C, 45.83; H, 4.61.

[1] IR spectra were run in potassium bromide using a Hitachi 215 spectrophotometer. UV spectra were recorded with a Shimadzu UV-300 spectrophotometer. PMR spectra were obtained, unless otherwise stated, in deuterated dimethyl sulfoxide on a Hitachi R-24B (60 MHz) spectrometer with chemical shifts measured relative to tetramethylsilane. Elemental analyses were carried out at the Central Analysis Center, Nagoya City University, Japan. Preparative TLC was conducted on Merck precoated TLC plates (silica gel 60 F254, 0.5- or 2 mm thick). Visualization was done with iodine vapor. Melting points were determined on a heat block melting-point apparatus and are uncorrected.

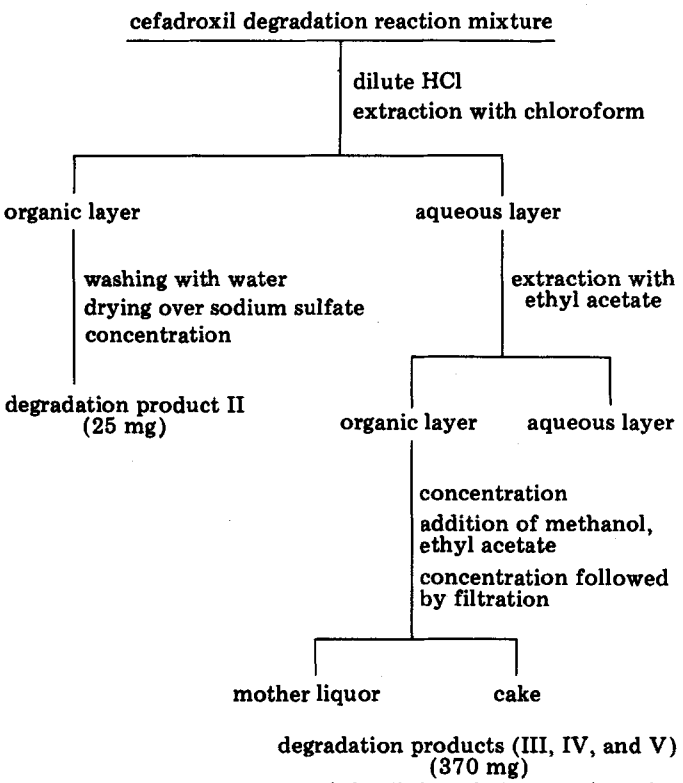
[2] pH-Stat titrator assembly consisting of TTT2 titrator and ABU12b autoburet, Radiometer, Copenhagen, Denmark, and PHM26 pH meter, Radiometer, Copenhagen, Denmark.

[3] Model FLC-A700, Japan Spectroscopic Co., Tokyo, Japan.

[4] Model UVIDEC-100, Japan Spectroscopic Co., Tokyo, Japan.

[5] μBondapak C_{18}, Waters Associates, Milford, Mass.

[6] Double-beam spectrophotometer, UV-200, Shimadzu, Kyoto, Japan.



Scheme II—Fractionation of cefadroxil degradation reaction mixture.

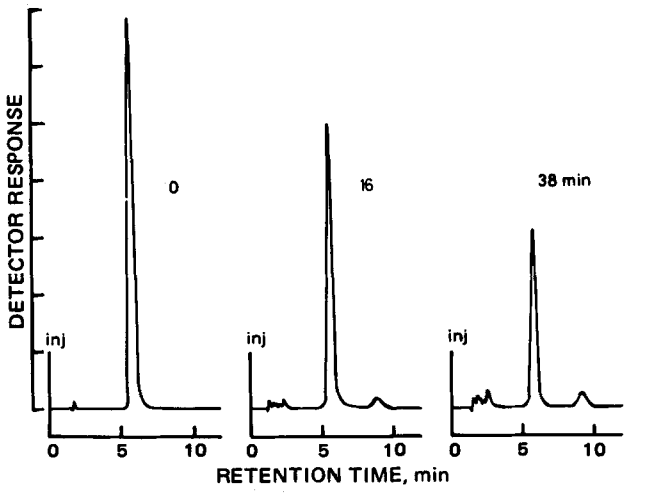
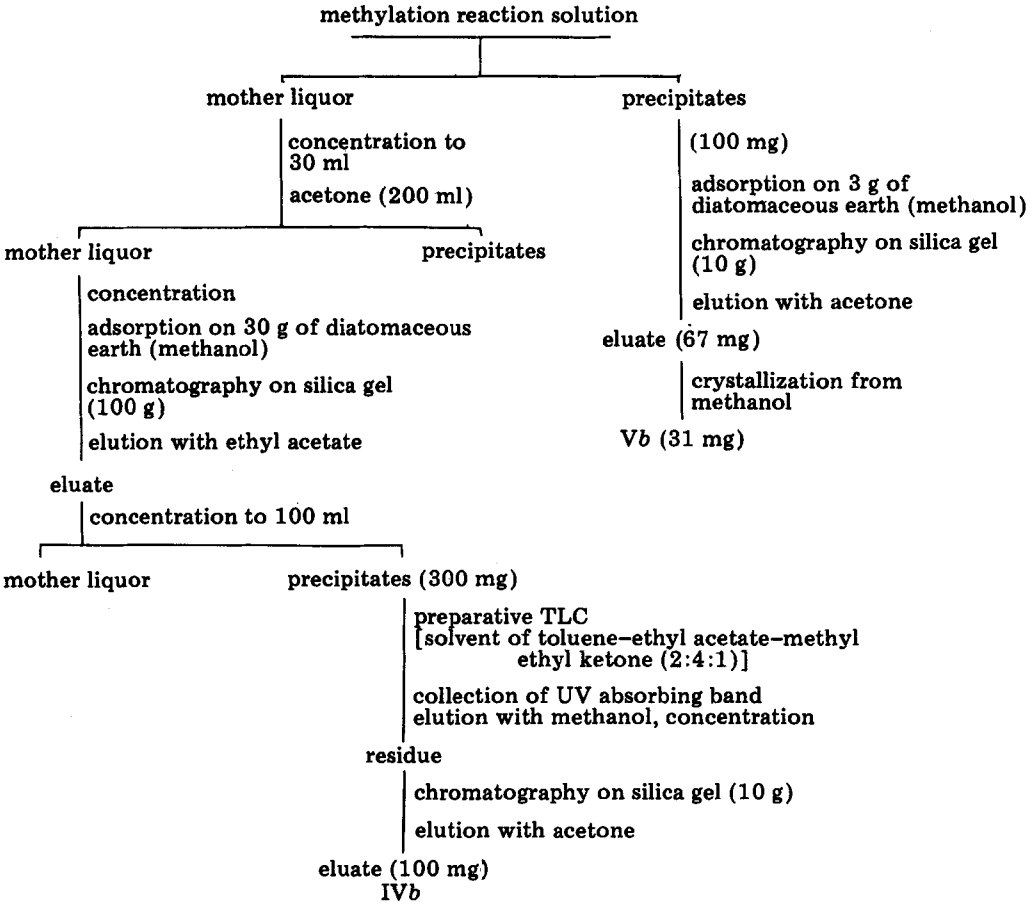


Figure 1—*Changes in the high-pressure liquid chromatogram during cefadroxil degradation at pH 11.50, 35°, and ionic strength 0.5 with time. The mobile phase was 2% acetonitrile–98% 0.01 M ammonium acetate, and samples were eluted at a flow rate of 2 ml/min.*

Mixture of Products of IV and V—IR: 3500–3000 (OH, NH), 1710 (shoulder, carboxyl), and 1660 (amide) cm^{-1}; PMR (d_5-pyridine): δ 1.43 (d, 8 Hz) and 2.41 (s) ppm (~2:1); PMR (d_5-pyridine–deuterium oxide): δ 1.44 (s) and 2.45 (s) ppm; PMR (deuterium oxide–potassium carbonate): δ 1.21 (s) ppm; UV (methanol): λ_{max} 225 and 273 nm; TLC: R_f (Solvent D) 0.62 (III, trace), 0.55 (IV), and 0.45 (V).

Degradation Products in Organic Solvent System—A suspension of cefadroxil (10 g) in methanol (250 ml) was refluxed for 3 hr while acetonitrile (100 ml) was added dropwise. Evaporation of the solvent *in vacuo* followed by the addition of chloroform (400 ml) to the residue yielded a precipitate (7.6 g), which was identified by IR and TLC as the mixture of IV and V slightly contaminated with III.

Methyl Esters of Products IV and V—The mixture of IV and V (7.5 g) was dissolved in warm methanol. After cooling, an ethereal solution (150 ml) of diazomethane, generated from *N*-methyl-*N*-nitroso-*p*-tolu-



Scheme III—Fractionation of the methylation reaction solution of cefadroxil degradation products.

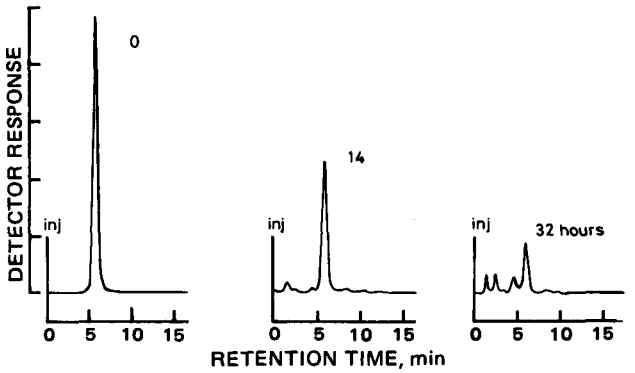


Figure 2—*Changes in the high-pressure liquid chromatogram during cefadroxil degradation at pH 7.20 (0.15 M phosphate), 35°, and ionic strength 0.5 with time. The mobile phase was 2% acetonitrile–98% 0.01 M ammonium acetate, and samples were eluted at a flow rate of 2 ml/min.*

enesulfonamide (30 g), was added dropwise over 1 hr. The precipitate (2.2 g) was filtered, and a part (100 mg) was chromatographed on silica gel (10 g) with acetone as the eluent to give pure ester V*b* (67 mg), mp 212–213°; IR: 3500–3000 (OH, NH), 1730 (ester), and 1680 (amide) cm^{-1}; PMR: δ 1.20 (d, J = 7 Hz, 3H), ~3 (m, 3H), 3.80 (s, 3H), 4.05 (m, 1H), 5.03 (s, 1H), 5.41 (m, 1H), 6.78 (d, J = 9 Hz, 4H), 7.85 (d, 1H, exchangeable), 8.38 (s, 1H, exchangeable), and 9.31 (b, 1H, exchangeable) ppm; UV: λ_{max} 226 and 276 nm.

Anal.—Calc. for $C_{17}H_{19}N_3O_5S$: C, 54.10; H, 5.07; N, 11.13. Found: C, 53.98; H, 5.00; N, 10.68.

The filtrate was separated as shown in Scheme III to give a small amount of ester IV*b*. Recrystallization from methanol yielded 100 mg of the pure ester, mp 214–215°; IR: 3500–3000 (OH, NH), 1720 (ester), and 1660 (amide) cm^{-1}; PMR: δ 2.04 (s, 3H), 3.05–3.5 (m, 2H), 4.25–4.6 (m, 2H), 4.85 (s, 1H), 6.55–7.3 (d, J = 9 Hz, 4H), 8.21 (s, 1H, exchangeable), 8.71 (s, 1H, exchangeable), and 9.37 (b, 1H, exchangeable) ppm; UV (methanol): λ_{max} 230 and 277 nm.

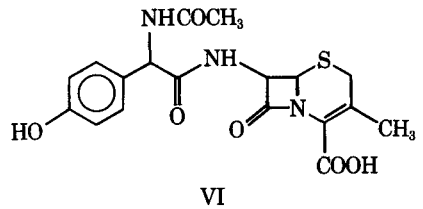
Anal.—Calc. for $C_{17}H_{19}N_3O_5S$: C, 54.10; H, 5.07; N, 11.13. Found: C, 53.53; H, 5.05; N, 10.93.

***N*-Acetylcefadroxil (VI)**—A suspension of cefadroxil (3 g) in acetic anhydride (200 ml) was stirred at ambient temperature for 15 hr. The precipitate was filtered and washed with water to yield pure VI (1.7 g, 53.3%) after recrystallization from methanol, mp 217–220° (dec.); IR: 3500–3000 (OH, NH), 1765 (β-lactam), 1735 (shoulder, carboxyl), and 1655 (amide) cm^{-1}; PMR: δ 1.88 (s, 3H, acetyl), 1.98 (s, 3H, 3-methyl), 3.0–3.7 (dd, J = 18 Hz, 2H, 2-CH_2), 4.93 (d, J = 5 Hz, 1H, 6-CH), 5.4–5.7 (broad, 3H, benzylic and 7-CH), 6.5–7.4 (dd, J = 8 Hz, 4H, aromatic), 8.35 (d, J = 9 Hz, 1H, 7-NH), 9.07 (d, J = 9 Hz, 1H, NH of acetamide), and 9.3 (broad, 1H, phenolic) ppm; UV (water): λ_{max} 228 and 263 nm.

Anal.—Calc. for $C_{18}H_{19}N_3O_6S$: C, 53.33; H, 4.72; N, 10.36. Found: C, 52.84; H, 4.33; N, 9.94.

RESULTS AND DISCUSSION

Order of Degradation and Observed Rate Constants—The kinetics of cefadroxil degradation was followed by the HPLC method using an appropriate buffer system or a pH-stat to maintain a constant pH. Typical chromatograms of basic (pH 11.50, pH-stat) and neutral (pH 7.20, 0.15 *M* phosphate buffer) reaction mixtures, sampled at suitable time intervals, are shown in Figs. 1 and 2, indicating that the degradation products can be separated from the parent compound. Figure 3 shows the logarithmic plots of the residual cefadroxil concentration *versus* time at pH 11.50 for three different initial concentrations of 1, 5, and 10×10^{-3} *M*. The observed pseudo-first-order rate constants were almost identical to the overall degradation of cefadroxil calculated from the slopes and were independent of initial drug concentration.



VI

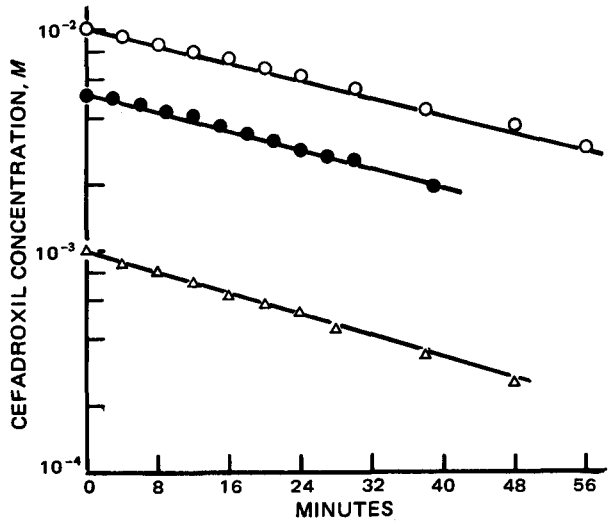


Figure 3—*Apparent first-order plots for cefadroxil degradation at three different concentrations (○, 1×10^{-2} M; ●, 5×10^{-3} M; and △, 1×10^{-3} M) at pH 11.50, 35°, and ionic strength 0.5, determined by HPLC assay.*

Similar semilogarithmic plots of the percent residual cefadroxil *versus* time were reasonably linear under various pH conditions (Fig. 4). These results indicate that the degradation of cefadroxil at constant pH, temperature, and ionic strength follows simple first-order kinetics with respect to cefadroxil. The observed rate constants and the buffer systems are listed in Table I.

Catalytic Effect of Buffer Systems—The catalytic effect of the two buffer systems used in the kinetic studies was determined at constant pH, temperature (35°), ionic strength (μ = 0.5), and drug concentration (5×10^{-3} *M*); only the buffer concentration was varied. This experiment was done at several pH values within the effective range for each buffer employed. The results (Figs. 5 and 6) show that the observed first-order rate constants increased linearly with an increase in the buffer concentrations at a constant pH in all cases.

Figure 5 shows the catalytic effect of citrate buffers between pH 2.51 and 5.82. The observed rate constant in the citrate buffer was actually a summation of several catalytic rate constants catalyzed by the buffer species plus the rate constant at zero buffer concentration. Accordingly, the observed rate constants, k_{obs}, may be expressed by:

$$k_{obs} = k_{pH} + k_{H_3A}[H_3A] + k_{H_2A^-}[H_2A^-] + k_{HA^{2-}}[HA^{2-}] + k_{A^{3-}}[A^{3-}] \quad \text{(Eq. 1)}$$

where k_{pH} represents the rate constant at zero buffer concentration; other k values are the second-order rate constants imposed by citrate buffer; $[H_3A]$, $[H_2A^-]$, $[HA^{2-}]$, and $[A^{3-}]$ are the concentrations of undissociated citric acid, dihydrogen citrate ion, monohydrogen citrate ion, and citrate ion, respectively.

The total citrate concentration, $[Cit]_T$, is shown as:

$$[Cit]_T = [H_3A] + [H_2A^-] + [HA^{2-}] + [A^{3-}] \quad \text{(Eq. 2)}$$

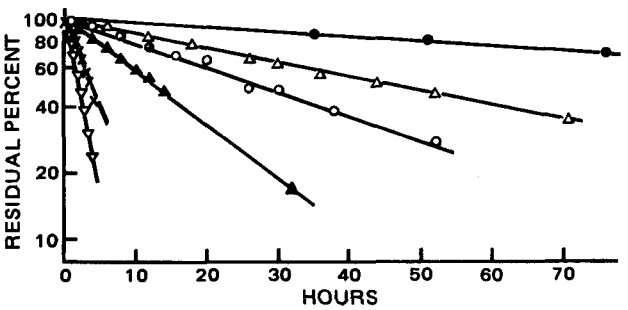


Figure 4—*Apparent first-order plots for the degradation of 5×10^{-3} M cefadroxil at various pH values, 35°, and ionic strength 0.5, determined by HPLC assay. Key: ●, pH 5.82 (0.11 M citrate buffer); △, pH 6.00 (0.10 M phosphate buffer); ○, pH 6.00 (0.2 M phosphate buffer); ▲, pH 7.20 (0.15 M phosphate buffer); ×, pH 10.50 (pH-stat); and ▽, pH 10.75 (pH-stat).*

Table I—Effects of Buffer Concentration and pH on the Pseudo-First-Order Rate Constants for Cefadroxil Degradation at 35° and Ionic Strength 0.5

pH (Buffer)	$10^3 k_{obs}$, hr^{-1}				$10^3 k_{pH}$, hr^{-1}
	0.05 *M*	0.10 *M*	0.15 *M*	0.20 *M*	
2.51 (Citrate)	0.937	0.987	1.03	0.979	0.950
3.11 (Citrate)	1.06	1.03	1.13	1.18	0.950
3.71 (Citrate)	1.10	1.25	1.34	1.48	0.950
4.31 (Citrate)	1.28	1.58	1.92	2.17	0.970
4.91 (Citrate)	1.69	2.33	2.99	3.58	1.02
5.82 (Citrate)	2.64 (0.037 *M*)	3.81 (0.073 *M*)	4.98 (0.110 *M*)	6.09 (0.146 *M*)	1.47
6.00 (Phosphate)	8.27	14.3	21.8	25.5	1.50
6.58 (Phosphate)	16.6	26.8	37.8	48.7	4.50
7.00 (pH-stat)	—	—	—	—	6.52
7.00 (pH-stat)	—	—	—	—	37.1 (50°)
7.00 (pH-stat)	—	—	—	—	92.6 (60°)
7.20 (Phosphate)	15.5 (0.03 *M*)	25.7 (0.06 *M*)	40.3 (0.10 *M*)	55.7 (0.15 *M*)	7.50
8.46 (pH-stat)	—	—	—	—	16.0
9.00 (pH-stat)	—	—	—	—	22.0
9.70 (pH-stat)	—	—	—	—	60.1
10.50 (pH-stat)	—	—	—	—	177
10.75 (pH-stat)	—	—	—	—	361
11.50 (pH-stat)	—	—	—	—	590 (30°)
11.50 (pH-stat)	—	—	—	—	1470
11.50 (pH-stat)	—	—	—	—	2180 (40°)
11.50 (pH-stat)	—	—	—	—	7560 (50°)

The dissociation constants of citric acid can be written as:

$$K_1^{cit} = \frac{[H_2A^-]a_{H^+}}{[H_3A]} \quad \text{(Eq. 3)}$$

$$K_2^{cit} = \frac{[HA^{2-}]a_{H^+}}{[H_2A^-]} \quad \text{(Eq. 4)}$$

$$K_3^{cit} = \frac{[A^{3-}]a_{H^+}}{[HA^{2-}]} \quad \text{(Eq. 5)}$$

From Eqs. 1–5, the following overall rate expression, k_{obs}, was obtained:

$$k_{obs} = k_{pH} + [Cit]_T \frac{k_{H_3A}(a_{H^+})^3 + k_{H_2A^-}K_1^{cit}(a_{H^+})^2 + k_{HA^{2-}}K_1^{cit}K_2^{cit}a_{H^+} + k_{A^{3-}}K_1^{cit}K_2^{cit}K_3^{cit}}{(a_{H^+})^3 + K_1^{cit}(a_{H^+})^2 + K_1^{cit}K_2^{cit}a_{H^+} + K_1^{cit}K_2^{cit}K_3^{cit}} \quad \text{(Eq. 6)}$$

In this case, no attempt was made to elucidate the catalytic constants with respect to the protonated and zwitterionic species of cefadroxil. By using the dissociation constants of citric acid, $pK_1^{cit} = 3.08$, $pK_2^{cit} = 4.75$, and $pK_3^{cit} = 5.40$ (11), the citrate catalytic constants at 35° were found from Eq. 6 and the slopes in Fig. 5 to be $k_{H_3A} = 9.70 \times 10^{-5}\ M^{-1}\ hr^{-1}$, $k_{H_2A^-} = 1.85 \times 10^{-3}\ M^{-1}\ hr^{-1}$, $k_{HA^{2-}} = 1.86 \times 10^{-2}\ M^{-1}\ hr^{-1}$, and $k_{A^{3-}} = 9.47 \times 10^{-2}\ M^{-1}\ hr^{-1}$.

Figure 6 shows the phosphate buffer effect between pH 6.00 and 7.20. Within this pH range, only the mono- and dihydrogen phosphate ions are significant. The overall rate expression can be given for the phosphate buffer effect as:

$$k_{obs} = k_{pH} + [Ph]_T \frac{k_{H_2PO_4^-}a_{H^+} + k_{HPO_4^{2-}}K_2^{Ph}}{a_{H^+} + K_2^{Ph}} \quad \text{(Eq. 7)}$$

where $k_{HPO_4^{2-}}$ and $k_{H_2PO_4^-}$ are the catalytic rate constants by mono- and dihydrogen ion species of phosphate and $[Ph]_T$ is the total phosphate concentration. By using the dissociation constant of the dihydrogen phosphate ion, $pK_2^{Ph} = 6.59$ at 35°, $\mu = 0.5$ (12), the catalytic rate constants on the degradation of cefadroxil were obtained from Eq. 7 and the slopes in Fig. 6 to be $k_{H_2PO_4^-} = 6.80 \times 10^{-2}\ M^{-1}\ hr^{-1}$ and $k_{HPO_4^{2-}} = 0.378\ M^{-1}\ hr^{-1}$.

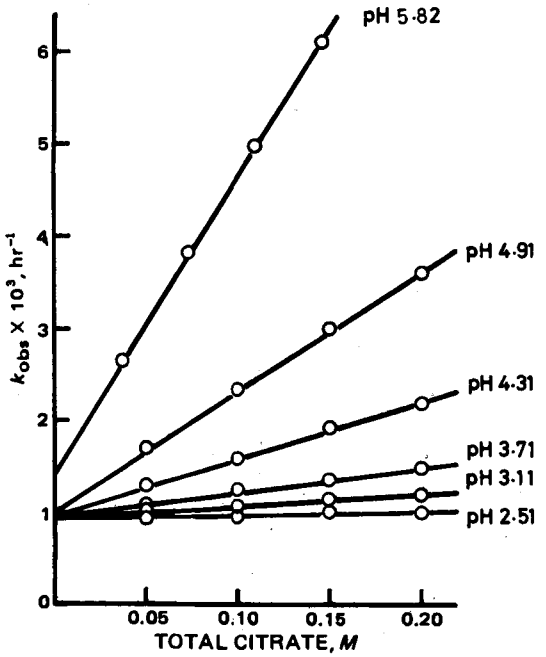


Figure 5—*Plots of pseudo-first-order rate constant* versus *total citrate buffer concentration for cefadroxil degradation at various pH values, 35°, and ionic strength 0.5.*

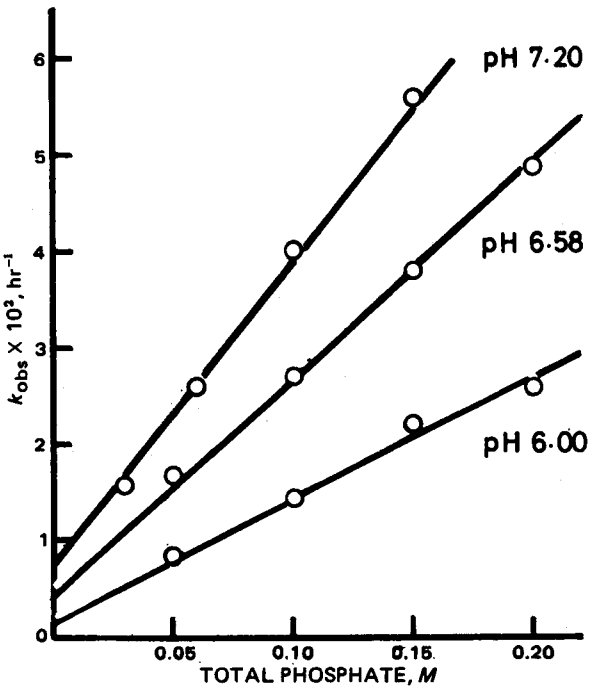


Figure 6—*Plots of pseudo-first-order rate constant* versus *total phosphate buffer concentration for cefadroxil degradation at various pH values, 35°, and ionic strength 0.5.*

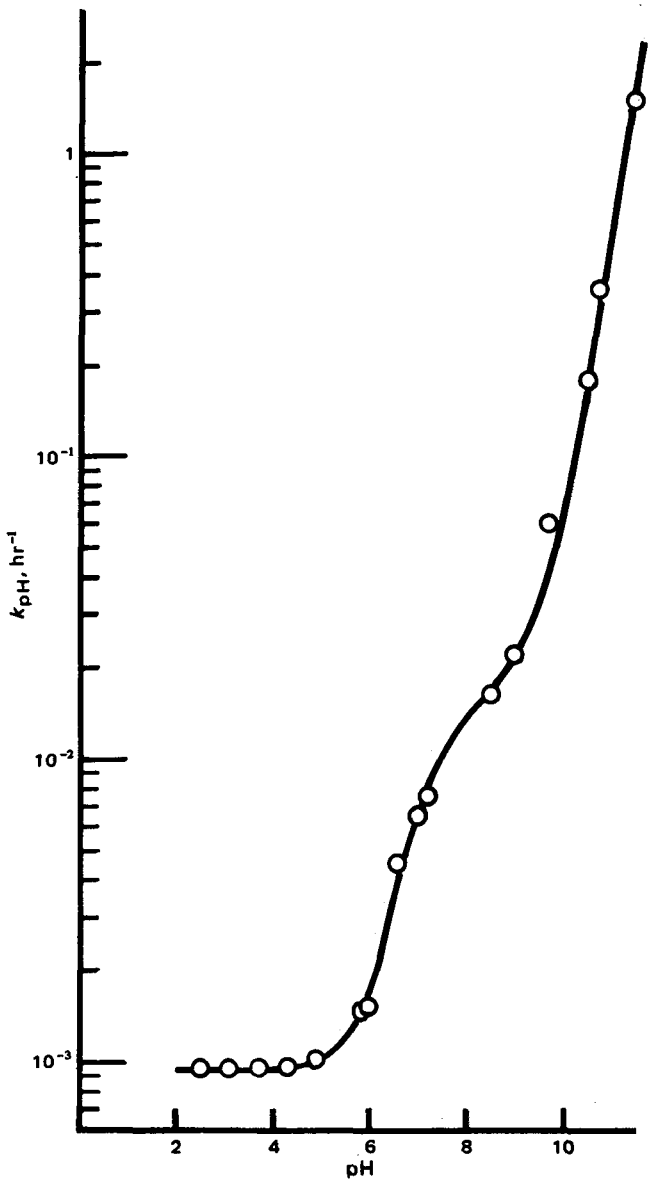


Figure 7—*Log k_{pH}–pH profile for cefadroxil degradation in aqueous solution at 35° and ionic strength 0.5, where k_{pH} is the apparent first-order rate constant for the degradation in buffer-free solutions or in buffers showing no effect on the degradation rate. The line represents the curve calculated from Eq. 10 and the constants in Table II; the points are experimental values.*

pH–Rate Profile—The pH dependence of the overall first-order rate constant, k_{pH}, of cefadroxil degradation at 35° and ionic strength 0.5 is shown in Fig. 7. The rate constants used in construction of the graph were obtained from the intercepts of the graphs of k_{obs} *versus* total buffer concentrations at various pH values (Figs. 5 and 6). The results from runs performed with a pH-stat were incorporated (Table I).

In the pH range studied, the amphoteric antibiotic cefadroxil exists in four different ionic forms: as a cation (IH_2^+), a zwitterion ($IH^\pm$), an anion (I^-), and a dianion (I^{2-}), the apparent pKa values of IH_2^+, $IH^\pm$, and I^- being 2.64, 7.30, and 9.69, respectively (35°, μ = 0.5) (Scheme I).

Figure 7 shows that at pH > 10, the observed rate of the degradation increased rapidly and uniformly with increasing pH. Since the slope of this straight-line portion of the log k_{pH} *versus* pH profile is unity, the dissociation of the phenolic moiety apparently has no effect on the β-lactam cleavage, and the specific hydroxide-ion-catalyzed reactions of anionic and dianionic cefadroxil take place at the same rate in this pH region and account exclusively for the total cefadroxil degradation.

At pH values near pKa_2, there was an ascending sigmoid dependence of k_{pH} on pH, followed by a rapid rate decrease as the pH decreased. This inflection indicates that the dissociation equilibria of the side-chain amino group influenced the degradation rate. Below pH 5, the degradation rate of cefadroxil was pH independent and not influenced by the dissociation of the 4-carboxylic acid group (pKa_1 = 2.64). This observation implies that the overall degradation consists chiefly of the water-catalyzed reactions of both cationic and zwitterionic cefadroxil. The hydrogen-ion-catalyzed cefadroxil degradation was negligible compared to the water-catalyzed reaction above pH 2.5.

The total shape of the log k_{pH}–pH profile of cefadroxil is very similar to that for cephalexin or cephradine (1) and suggests that the rate expression given in Eq. 8 also holds true for the overall degradation of cefadroxil:

$$\frac{d[I]_T}{dt} = -[k_0(f_{IH_2^+} + f_{IH^\pm}) + (k_b + k_{OH}K_w/a_{H^+})(f_{I^-} + f_{I^{2-}})][I]_T \quad \text{(Eq. 8)}$$

where k_0 represents the first-order rate constant for water-catalyzed degradation of both cationic and zwitterionic cefadroxil; k_b represents the first-order rate constant for the spontaneous (or water-catalyzed) degradation of the ionic and dianionic species of cefadroxil; k_{OH} represents the second-order rate constant for specific hydroxide-ion-catalyzed degradation of all anionic species; K_w is the autoprotolytic constant; $f_{IH_2^+}$, $f_{IH^\pm}$, f_{I^-}, and $f_{I^{2-}}$ represent the mole fractions of the cationic, zwitterionic, anionic, and dianionic species of amphoteric cefadroxil, respectively; and $[I]_T$ is the total cefadroxil concentration.

By introducing the first-order expression:

$$-\frac{d[I]_T}{dt} = k_{pH}[I]_T \quad \text{(Eq. 9)}$$

the following pseudo-first-order rate expression is derived since only the dissociation of the 7-side chain amino group could influence the degradation rate:

$$k_{pH} = k_0\frac{a_{H^+}}{a_{H^+} + K_{a_2}} + (k_b + k_{OH}K_w/a_{H^+})\frac{K_{a_2}}{a_{H^+} + K_{a_2}} \quad \text{(Eq. 10)}$$

The theoretical degradation rate constant of cefadroxil was calculated for the pH of 2.5–11.5 range. By employing $K_w = 2.09 \times 10^{-14}$ at 35° (13), the various rate constants listed in Table II were best satisfied. In Fig. 7, the line represents the theoretical curve calculated by substituting these k values into Eq. 10 while the points show the experimental results. The good agreement indicates that this equation adequately describes the kinetics of total cefadroxil degradation.

Degradation Products in Neutral and Alkaline Solutions—The thin-layer chromatogram of the degradation reaction solution of cefadroxil, conducted in a basic phosphate buffer at pH 9.1 at 60° for 1 hr, revealed four spots other than cefadroxil. These spots were tentatively named products II, III, IV, and V according to their R_f values on TLC. Closer inspection of the chromatogram showed the major products to be IV and V.

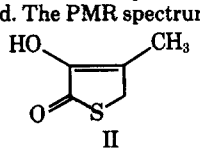
Products III, IV, and V also were detected in the degradation carried out in methanol–acetonitrile, which suggests that the main degradation process is almost identical in the two solutions, but no trace of II was observed.

Attempts to isolate and characterize the reaction mixture met with difficulties and proved only partially successful. Treatment of the mixture as shown in Scheme II yielded II and a mixture of IV and V slightly contaminated with III. Further separation of the latter mixture under repetitive chromatography was successful.

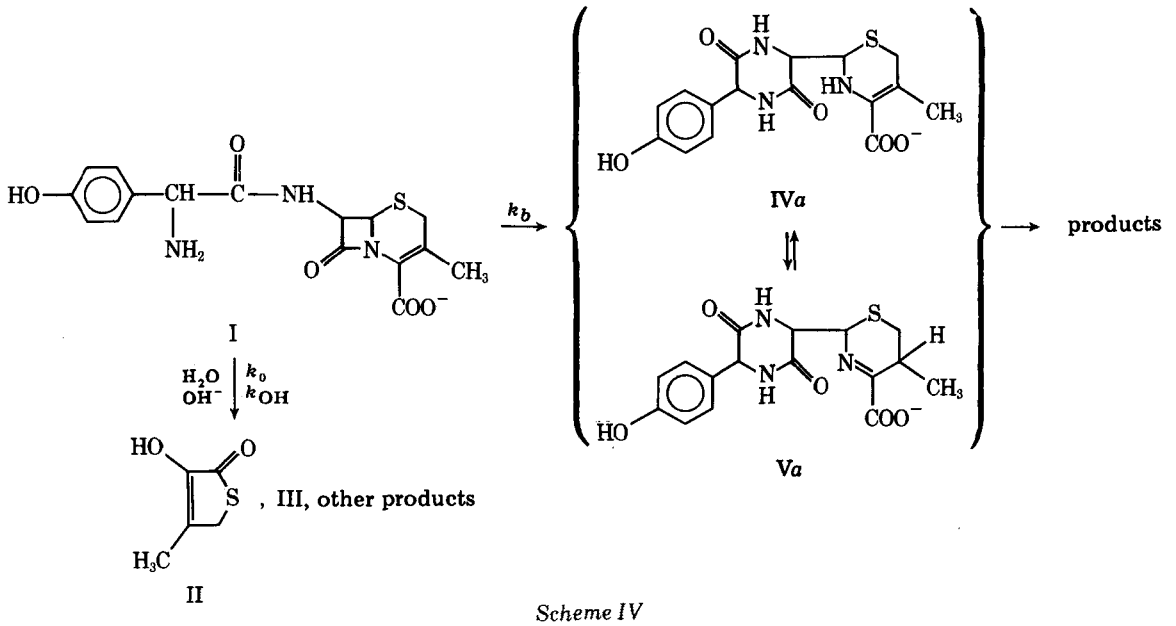
The structural elucidation of II was achieved based on spectral data. The PMR spectrum revealed simple patterns composed of three singlet signals at δ 2.09, 3.74, and 6.10 ppm, with signal areas in the ratio of 3:2:1, respectively. Furthermore, the IR spectrum exhibited three characteristic absorptions at 3370, 1690, and 1630 cm^{-1}, which suggests the presence of an enol lactone. The UV spectrum showed absorbances at 246 and 255 nm, the latter showing a typical bathochromic shift to 304 nm on the addition of a base. This finding led to the determination of II as the thiol lactone, 3-hydroxyl-4-methyl-2-(5*H*)-thiophenone. Elemental analysis data supported the structure.

Compound II is reported in the literature as the degradation product of cephalexin in experiments conducted at pH 7.4 (6) and 3.3 (7).

The mixture of IV and V, the major products, displayed no peaks corresponding to a β-lactam in its IR spectrum, strongly indicating that the β-lactam was cleaved. The PMR spectrum in d_5-pyridine revealed,



II



Scheme IV

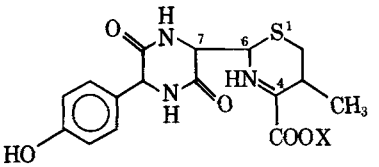
among others, two signals ascribable to methyl groups at δ 1.58 (doublet) and 2.23 (singlet) ppm, the approximate ratio of the integrated signal areas being 2:1. This finding suggests the existence of two different methyl groups in the mixture, most likely one attached to a secondary carbon atom and the other attached to a double bond. Moreover, since the mixture showed no reaction with dinitrobenzenesulfonic acid, the lack of free amino groups is evident. From these results, it was speculated that the mixture is composed of the piperazinedione derivatives, IV*a* and V*a*.

Further information on the structure of the components was gained by derivatizing the mixture to the respective methyl esters with diazomethane in methanol. Chromatographic separations as shown in Scheme III proved successful in isolating each derivative.

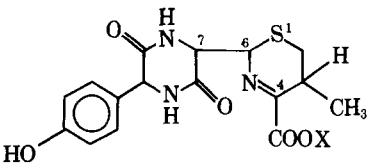
The PMR spectrum of the major product, V*b*, showed a doublet signal at δ 1.20 ppm ascribable to a methyl group on a secondary carbon atom coupling with a vicinal proton. On addition of deuterium oxide, the signals centered at δ 4.05 and 5.41 ppm showing complex coupling patterns simplify, making it reasonable to assign the former to the proton on C-7 and the latter to the proton on C-6 showing longrange coupling with protons on C-2 and/or C-3. The IR spectrum revealed peaks for ester carbonyl (1730 cm^{-1}) and superimposed amide carbonyls (1680 cm^{-1}), while the UV spectrum showed absorbances at 226 and 276 nm, assigned to the phenol group. All data proved to be consistent with the proposed structure, a piperazinedione derivative with the double bond at the C-4, C-5-position.

The minor product, IV*b*, exhibited IR and UV spectra closely resembling those of V*b*. The PMR spectrum revealed a singlet at δ 2.04 ppm, which was the decisive factor in determining its structure as the piperazinedione derivative with the double bond at the C-3, C-4-position.

Elemental analyses for the two esters supported the illustrated structures. The characterization of the methyl derivatives led to the conclusion that the major degradation products of cefadroxil in basic aqueous solution have structures IV*a* and V*a*, as illustrated in Scheme IV.



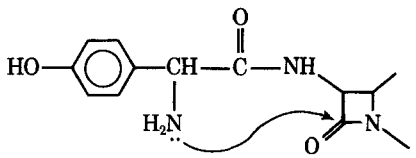
V*a*: X = H
V*b*: X = CH_3



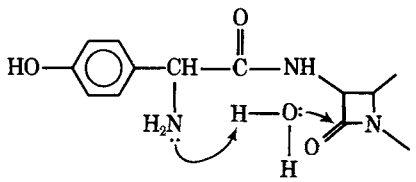
IV*a*: X = H
IV*b*: X = CH_3

Mechanism of Reaction Involving Side-Chain α-Amino Group—The shape of the pH–rate profile of cefadroxil in the neutral pH region may be accounted for by the following three mechanisms (Scheme V): (*a*) intramolecular nucleophilic attack of unprotonated side-chain amino groups on the β-lactam carbonyl moiety, (*b*) intramolecular general base catalysis by the amino group inducing the attack of a water molecule on the β-lactam moiety, and (*c*) intramolecular general acid catalysis by the protonated amino group inducing the attack of hydroxide ion on the β-lactam moiety. These mechanisms are kinetically indistinguishable.

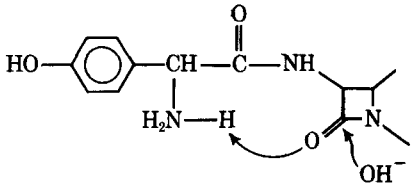
Mechanism *a* is markedly different from the other two in the formation of piperazine-2,5-dione products, whose structures are shown here as IV*a* or V*a*. Cohen *et al.* (5) reported the isolation of a piperazinedione product similar to V*a* from an aqueous sodium carbonate solution of cephradine. Recently, the piperazinedione was isolated from an aqueous phosphate solution of cephalexin kept at 35° and pH 7.6 for 24 hr (6). In this study, the structural speculation of the mixture of IV*a* and V*a* for the precipitated products from the basic solution of cefadroxil and the successful



Mechanism a



Mechanism b



Mechanism c

Scheme V

Table II—Dissociation Constants and Rate Constants [a] for the Degradation of Aminocephalosporins at 35° and Ionic Strength 0.5

Antibiotic	pKa_1	pKa_2	$10^3 k_0$, hr^{-1}	$10^2 k_b$, hr^{-1}	$10^{-2} k_{OH}$, $M^{-1} hr^{-1}$
Cefadroxil	2.64	7.30	0.941	1.61	2.54
Cephalexin[b]	2.56	6.88	1.15	1.01	2.64
Cephradine[b]	2.53	7.30	1.10	0.740	3.98
Cephaloglycin[b]	1.91	6.90	5.00	13.5	13.1

[a] Rate constants are defined in Eq. 10. [b] Reference 1.

isolation and identification of their methyl esters (IV*b* and V*b*) supported the occurrence of Mechanism *a*.

Additional evidences for the intramolecular aminolysis reaction (Mechanism *a*) were obtained. Figure 8 shows the first-order plots of the disappearance of amino groups by following the decrease in the concentration of primary amino groups in cefadroxil solution during its degradation in 0.15 *M* phosphate buffer at pH 7.20, 35°, and $\mu = 0.5$, the plots being obtained by the Guggenheim method (14). Total disappearance followed by the HPLC method for the same samples is also shown in Fig. 8. The apparent first-order rate constants obtained from the two methods are in reasonable agreement, suggesting that the loss of cefadroxil is accompanied by a parallel loss of its amino group. Furthermore, when *N*-acetylcefadroxil (VI) was heated in a neutral phosphate buffer, no reaction was observed on TLC, indicating that the degradation required a free amino group attacking the β-lactam moiety.

These results lead to the conclusion that the degradation of cefadroxil proceeding at neutral and basic pH was dominated by a spontaneous aminolysis (Mechanism *a*) rather than water-catalyzed aminolysis to produce the piperazinediones IV*a* and V*a*.

Equilibrium between Degradation Products—PMR spectral studies of the mixture of IV*a* and V*a* suggested the existence of an equilibrium between them. PMR spectra in d_5-pyridine showed a doublet at δ 1.21 ppm which, on addition of deuterium oxide, changed to a broad singlet; no change was observed with the singlet at δ 2.01 ppm, clearly indicating the presence of an equilibrium shown in Scheme IV where the C-3 proton was easily exchanged by deuterium oxide through the migration of the double bond. Further evidence for the equilibrium was obtained when the degradation of cefadroxil in potassium carbonate–deuterium oxide solution was monitored by PMR. The spectrum showed the singlet at δ 2.01 ppm, assigned to the methyl group of cefadroxil, gradually disappearing with time while a new singlet assignable to a secondary methyl group appeared. This change indicates the double bond migration, probably to the 4,5-position, to predominantly yield V*a* and exhibits the same behavior found for cephradine (5) and cephalexin (1).

Similar migration of the double bond was also observed between the methyl esters, IV*b* and V*b*. In the PMR spectra of IV*b* in d_6-dimethyl sulfoxide, the addition of deuterium oxide and trifluoroacetic acid to the sample resulted in the appearance of new signal peaks, corresponding to the spectrum of V*b*, which increased in area until an apparent equilibrium was reached. Integration of peak areas showed that the equilibrium was attained at a ratio of 3:1 with IV*b* predominating.

This result was confirmed by conducting the same experiment, this time starting with V*b*, which showed the appearance and increase of small peaks corresponding to IV*b* until a similar equilibrium was reached in favor of IV*b*. Furthermore, data obtained from TLC of the esters in acidic conditions also supported the existence of an equilibrium.

Available Evidence for Formation of II and a Possible Degradation Pathway in Aqueous Solution—The degradation product II was isolated or confirmed from the cefadroxil degradation solutions in water and in 0.1 *M* dibasic sodium phosphate solution at pH 9.1. However, when cefadroxil was degraded in neutral organic solvents, no formation of II was observed on TLC. Bundgaard (6) reported the isolation of 3-hydroxy-4-methyl-2(5*H*)-thiophenone (II) from the degradation solution of cephalexin in 0.5 *M* phosphate buffer at pH 7.5 and proposed

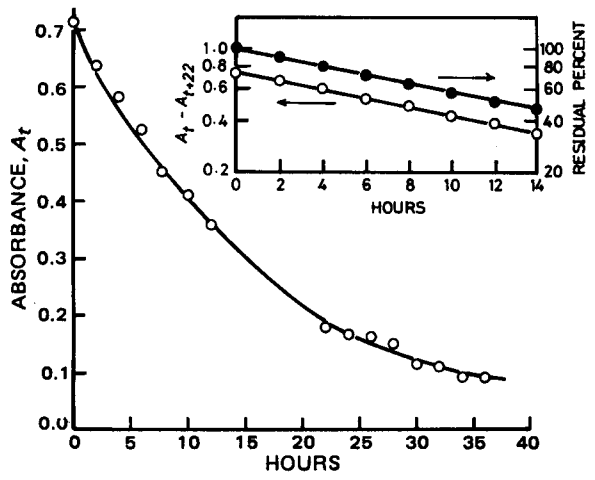


Figure 8—*Time courses for primary amino group disappearance during cefadroxil (5×10^{-3} M) degradation in 0.15 M phosphate buffer (pH 7.20) at 35° and ionic strength 0.5. Absorbance refers to the absorbances produced by subjecting equal aliquots of the reaction solution to the trinitrobenzenesulfonic acid assay. The inset shows the first-order plots of the data (○) obtained by Guggenheim treatment (22-hr intervals) in comparison with the HPLC assay (●) of the same samples.*

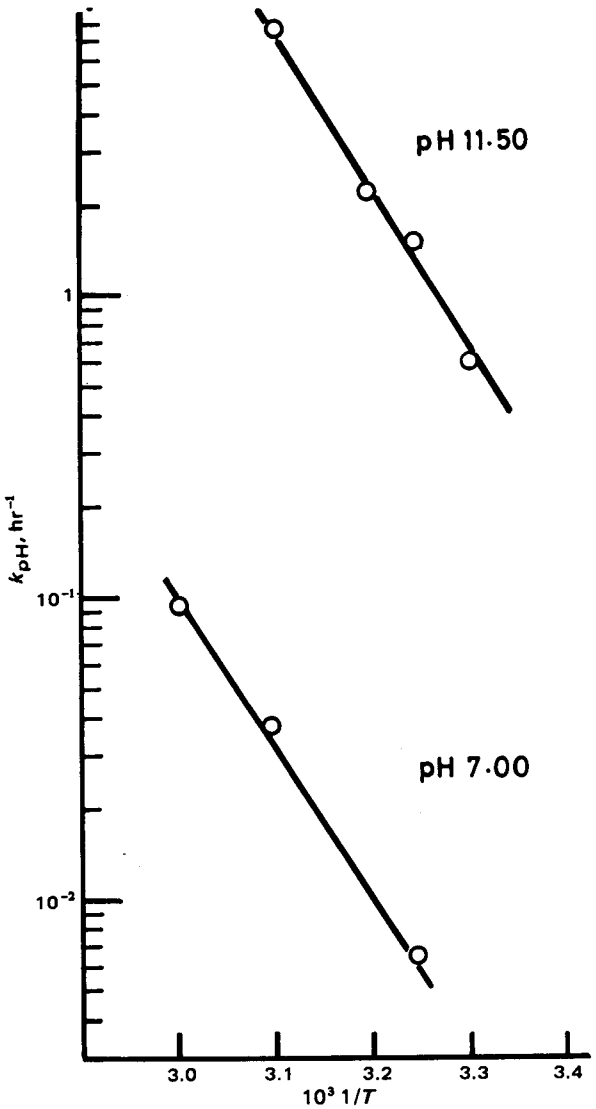


Figure 9—*Arrhenius plots for the apparent first-order rate constants, k_{pH}, for cefadroxil degradation at pH 7.00 and 11.50 and ionic strength 0.5. The pH values were maintained by a pH-stat.*

that this thiophene derivative might be transformed from the piperazinedione product.

To clarify the possible formation of II from IV*a* or V*a*, the product mixture IV*a* and V*a* was heated in an alkaline phosphate solution. Several secondary degradation products were detectable on TLC, but none had the same R_f value as II. The results suggest that product II does not form *via* IV*a* and V*a* but is directly formed from cefadroxil *via* a different pathway.

Product III was difficult to isolate in amounts sufficient to characterize it; its formation was confirmed in neutral and basic degradation solutions of cefadroxil.

A possible route to account for the observed formation of II, III, IV*a*, and V*a* is shown in Scheme IV.

Temperature Dependency—Rate constants for the overall disappearance of cefadroxil were obtained from 35 to 60° at pH 7.00 and 11.50. The pH of the reaction solution was maintained constant by the use of a pH-stat. The Arrhenius plots are shown in Fig. 9. From these data, the apparent activation energies at pH 7.00 and 11.50 was determined to be 21.4 and 23.8 kcal/mole, respectively.

In aqueous solution at pH 7.00 and 35°, cefadroxil degraded *via* intramolecularly catalyzed reaction (k_b reaction) to an extent of 83% and *via* a water-catalyzed β-lactam opening (k_0 reaction) to an extent of 17%.

At pH 11.50, the hydroxide-ion catalyzed degradation proceeded exclusively, the contribution of the k_b reaction being only 0.1%. The apparent activation energy of 23.8 kcal/mole at this alkaline pH may include the heat of ionization of water, 13.1 kcal/mole (13). The net activation enthalpy, $\Delta H^{\neq}$, of hydroxide-ion-catalyzed degradation of cefadroxil was calculated to be 10.5 kcal/mole at 35°.

Comparative Stability Among Aminocephalosporins—Table II lists various rate constants defined by Eq. 10 for the degradation of cephaloglycin, cephalexin, and cephradine determined previously under the same kinetic conditions. It is apparent that the stability pattern of cefadroxil resembles that of cephalexin and cephradine over all pH regions.

A comparison of the specific rate constants of k_0, k_b, and k_{OH} for the four aminocephalosporins shows that the β-lactam moiety of cephaloglycin, which has an acetoxymethyl group in the C-3 position, is about four to five times more susceptible to attack of both water and hydroxide ion and eight to 18 times more reactive to intramolecular attack by the C-7 side-chain amino group than that of cephalosporins possessing a methyl group at the C-3 position, *i.e.*, cefadroxil, cephalexin, and cephradine. The difference in reactivity between cephaloglycin and the other three antibiotics may be ascribed to the difference in the inductive ability of their C-3 substituents and/or leavability of the C-3 moiety, as suggested previously for cephalosporin degradations (1, 3, 15–18).

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Alterations in Integrity of Goldfish Membrane Induced by Edetate Disodium

P. J. CASCELLA ˣ, E. G. HUPPLER, II, and J. D. JOHNSON

Received October 20, 1980, from the *College of Pharmacy, South Dakota State University, Brookings, SD 57007.* Accepted for publication March 5, 1981.

Abstract □ The effect of the chelating agent edetate disodium on the integrity of the goldfish membrane was examined. The time to produce death in goldfish exposed to secobarbital sodium was used as a reflection of membrane integrity. Although a minimum edetate disodium concentration was necessary to induce alterations in integrity, no direct relationship between the effect and concentration of the chelating agent was evident. The chelating agent's effect appeared to be an enhancement of the transport of the ionized drug form. The change in membrane integrity existed at least 24 hr after theoretical exposure to edetate disodium, but cyclic alterations in integrity could not be ruled out. The effect on integrity was also demonstrated to be nonpermanent, and the apparent loss in integrity was partially restored by calcium but not by magnesium.

Keyphrases □ Permeability—effect of edetate disodium on goldfish membrane □ Edetate disodium—effect on membrane permeability, goldfish □ Chelating agents—edetate disodium, effect on membrane permeability, goldfish

Edetic acid is a chelating agent capable of changing transfer rates of certain substances across membrane barriers (1–4). It was reported (1) that the sodium salt of edetic acid was capable of enhancing the absorption of heparin and synthetic heparinoids from the GI tract of rats and dogs and that these results were consistent with another investigation (2) in the monkey, dog, cat, rabbit, and human. Feldman and Gibaldi (3) demonstrated that edetic acid was capable of affecting the transfer rate of salicylate, but not salicylamide, across the everted rat intestine. They concluded that edetic acid was capable of altering the barrier for water-soluble, but not lipid-soluble, compounds. It was also demonstrated (4) that the sodium salt of edetic acid was capable of increasing phenolsulfonphthalein absorption in rats.

Although edetic acid is capable of changing the permeability of membranes to various compounds, the exact nature of the mechanism involved is unknown. Regardless of the mechanism, numerous studies have been performed to see if the effects of the chelating agent can be reversed. Windsor and Cronheim (1) found that the magnesium and

0022-3549/81/1000-1128$01.00/0

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