JOURNAL OF PHARMACEUTICAL SCIENCES

OCTOBER 1979
VOLUME 68 NUMBER 10

RESEARCH ARTICLES

Degradation of Mecillinam in Aqueous Solution

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Received September 25, 1978, from *Leo Pharmaceutical Products, DK-2750 Ballerup, Denmark.* Accepted for publication April 10, 1979.

Abstract □ The hydrolysis of mecillinam in aqueous solution (37°) was studied at pH 2–10. The degradation products observed by TLC and NMR were identified and quantified. Several of these compounds were synthesized. Mecillinam and the key degradation product, (6*R*)-6-formamidopenicillanic acid, underwent reversible 6-epimerization in basic solution. Some of the thiazolidine derivatives formed epimerized at position 2. In contrast to penicillins, the degradation pattern of mecillinam becomes more complex with increasing pH. Rate constants for some processes are given.

Keyphrases □ Mecillinam—degradation in aqueous solution, isolation of degradation products, degradation pathways, rate constants □ Hydrolysis—mecillinam in aqueous solution, degradation products □ Antibiotics—mecillinam, degradation in aqueous solution, isolation of degradation products, degradation pathways, rate constants

Degradation of penicillins in aqueous solution has been studied for more than 30 years, and many decomposition products have been identified. Different degradation pathways prevail in acidic and basic solutions. Although the side-chain amide group of penicillin is discernible in most primary degradation products, their fundamental structures apparently are not influenced by the nature of the acyl group, even if it contains substituents, *e.g.*, the ampicillin amino group, seemingly in an ideal position for further reaction with the strained β-lactam ring. Only the reactivity of the β-lactam ring is influenced by inductive and steric effects of the side chain (1, 2). A few degradation products are exclusively derived from the 6-aminopenicillanic acid nucleus common to all penicillins.

Mecillinam (FL 1060), (6*R*)-6-[(hexahydro-1*H*-azepin-1-yl)methyleneamino]penicillanic acid (I) (Table I), represents a new type of β-lactam antibiotic having the side chain linked to a 6-aminopenicillanic acid moiety through an amidine bond. These derivatives have predominant activity against Gram-negative bacilli and only weak activity against Gram-positive organisms (3). In accordance with this unusual antibacterial spectrum, Gram-negative bacteria are killed by I differently than by penicillins and cephalosporins (3, 4).

A substituted amidinopenicillanic acid may be expected to differ from the penicillins in its hydrolytic degradation, because amidines generally are hydrolyzed more easily than amides (5). Furthermore, the hydrolysis produces amine(s) capable of reaction with the β-lactam ring. Amidines show great variation in their hydrolysis rate (6), and the nature of the amidinopenicillanic acid substituents may also be expected to influence the rate of other degradation reactions.

In the present study, I was chosen as a representative amidinopenicillanic acid. TLC and NMR methods identified and semiquantified the major degradation products formed in aqueous I solutions as a function of time and pH. Previous investigations in these laboratories[1] showed that I is more stable in acidic than in basic solution. Optimum stability (half-life ~200 hr at 37°) was found at around pH 5, not far from the isoelectric point of I (5.8). Similar results were reported recently from other laboratories (7, 8).

EXPERIMENTAL

Materials—Mecillinam (I) and the reference compounds listed in Table I, except VI, VIII, X, and XII, were synthesized[2]. None of the yields quoted was optimized. TLC and NMR data of VI, VIII, X, and XII were obtained from aqueous solutions of their analogs with natural configuration after equilibration. Penicic acid was prepared according to a literature method (9). All other chemicals were analytical grade.

Melting points are uncorrected. NMR chemical shifts are given in parts per million (delta scale) with tetramethylsilane or sodium 3-trimethylsilylpropanoate as the internal reference. IR data are given in centimeters^{-1}.

Mecillinam (I)—This compound was prepared according to production methods (10) in better than 99% purity as determined by iodometric titration, spectrophotometric assay, and TLC; $[\alpha]_D^{20}$ +294° (H_2O, pH 2.5)[3]; PMR[4] (CD_3OD, tetramethylsilane): 1.66 (s, 2αCH_3), 1.73 (s, 2βCH_3), 4.28 (s, 3H), 5.37 (d, J = 4 Hz, 6H), 5.56 (d, J = 4 Hz, 5H), 1.5–2.1 [m, $(CH_2)_4$], 3.5–3.9 (m, CH_2NCH_2), and 8.10 (s, NCH=N); IR[5] (0.3% KBr): 1770, 1672, and 1590.

[1] E. Gundersen, Leo Laboratories, personal communication.
[2] Leo Laboratories.
[3] Perkin-Elmer PE 141.
[4] Varian A60A (cw), Jeol PMX 60 (cw), and Jeol FX 100 (ft, 24K).
[5] Perkin-Elmer PE 457 grating spectrophotometer.

0022-3549/79/1000-1207$01.00/0
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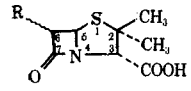
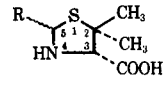
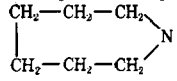
Table I—Structures of Mecillinam and Its Degradation Products

Compound[a]	R–[b]	Structure
I Mecillinam	(N—CH=N—	
II (6*S*)-Mecillinam	(N—CH=N—	
III 6-Formamidopenicillanic acid	OCH—NH—	
IV (6*S*)-6-Formamidopenicillanic acid	OCH—NH—	
V Mecillinam penicilloic acid	(N—CH=N—CH(—COOH)—	
VI (6*S*)-Mecillinam penicilloic acid	(N—CH=N—CH(—COOH)—	
VII Mecillinam penilloic acid	(N—CH=N—CH_2—	
VIII (5*S*)-Mecillinam penilloic acid	(N—CH=N—CH_2—	
IX Penicilloic acid	OCH—NH—CH(—COOH)—	
X (5*S*)-Penicilloic acid	OCH—NH—CH(—COOH)—	
XI Penilloic acid	OCH—NH—CH_2—	
XII (5*S*)-Penilloic acid	OCH—NH—CH_2—	
XIII Penicilloic amide	OCH—NH—CH(—CON)	

[a] Although not strictly correct, the nomenclature and numbering of penicillin are used for all degradation products including monocyclic compounds except in the *Experimental* part. When not otherwise indicated, the compounds have the natural penicillin configuration (3*S*, 5*R*, 6*R*). [b] (N stands for 

(6*S*)-6-[(Hexahydro-1*H*-azepin-1-yl)methyleneamino]penicillanic Acid (II)—*Isolation from Alkaline Degradation of I*—A I solution (60 g, 185 mmoles) in water (250 ml) was adjusted to pH 9.5 with 2 *N* NaOH and kept for 2.5 hr at 37° and pH 9.5, followed by treatment with 6,000,000 units of β-lactamase[6] (11) at constant pH 7 at room temperature. When no more 2 *N* NaOH was consumed (~1 hr), the pH was raised to 10 with hexamethyleneimine. The solution was extracted with ether (200 ml) and chloroform (2 × 800 ml), and the combined chloroform phases were dried and evaporated *in vacuo*.

The oily residue was dissolved in water (400 ml), the pH was adjusted to 5.5, and the solution was freeze dried. The product (12 g) was triturated with acetone (100 ml) for 0.5 hr. After filtration, the filtrate was evaporated *in vacuo* to leave a solid (11.2 g), which was dissolved in isopropanol (160 ml). Then 8 *N* hydrogen chloride in isopropanol (4.4 ml) and water (4 ml) was added, and the hydrochloride hydrate of II was precipitated (6.1 g) by the addition of isopropyl ether (300 ml).

Recrystallization from isopropanol–isopropyl ether yielded 3.8 g (5.5%) of the pure compound, mp 170–173°; $[\alpha]_D^{20}$ +248° (H_2O, pH 2.5); $[\alpha]_D^{20}$ +290° calculated as the anhydrous zwitterion; PMR (CD_3OD, tetramethylsilane): 1.53 (s, 2αCH_3), 1.62 (s, 2βCH_3), 1.5–2.1 [m, $(CH_2)_4$], 3.5–4.0 (m, CH_2NCH_2), 4.54 (s, 3H), 5.07 (d, J = 1.6 Hz, 6H), 5.43 (d, J = 1.6 Hz, 5H), and 8.23 (s, NCH=N); ^{13}C-NMR [$(CD_3)_2SO$, tetramethylsilane]: 32.7 (CH_3), 68.3 (C-3), 68.6 (C-5), 71.2 (C-6), 156.2 (NC=N), 166.8 (C-7), and 167.7 (COO); IR (0.3% KBr): 1774, 1723, and 1675.

Anal.—Calc. for $C_{15}H_{26}ClN_3O_4S$: C, 47.42; H, 6.90; N, 11.06; S, 8.44; H_2O, 4.73. Found: C, 47.69; H, 7.02; N, 11.00; S, 8.51; H_2O, 4.07.

Synthesis of Benzyl (6S)-6-[(Hexahydro-1H-azepin-1-yl)methyleneamino]penicillanate Nitrate—Benzyl (6*S*)-6-aminopenicillanate (12) (1.7 g, 5.5 mmoles) was stirred in hexamethyleneiminecarboxaldehyde dimethyl acetal (15 ml) for a few minutes. The resultant solution was kept at room temperature overnight, followed by stirring for 10 min at 0–5° with water (30 ml). The acidified reaction mixture (pH 2.5) was extracted with ether (30 ml) and subsequently with ether (2 × 30 ml) at pH 7.5.

The organic phase from the alkaline extraction was extracted with water (5 × 15 ml), dried, and evaporated *in vacuo* to leave an oil (2.5 g). To an isopropanolic solution (20 ml) of this oil was added a solution of concentrated nitric acid (0.3 ml) in isopropanol (3 ml), and the mixture was cooled to 0–5°. The precipitate (1.9 g) was recrystallized from methanol–ether to yield 1.4 g (53%) of the title compound, mp 172°; $[\alpha]_D^{20}$ +222° (96% C_2H_5OH); PMR [$(CD_3)_2SO$, tetramethylsilane]: 1.37 (s, 2αCH_3), 1.53 (s, 2βCH_3), 1.4–2.0 [m, $(CH_2)_4$], 4.71 (s, 3H), 5.11 (m, 6H), 5.22 (s, O$CH_2C_6H_5$), 5.33 (d, J = 2 Hz, 5H), 7.38 (s, C_6H_5), and 8.22 (bs, NCH=N); IR (0.3% KBr): 1775, 1748, 1682, 1382, 1202, 1180, 765, 752, 703, and 508.

[6] Cerius β-lactamase, Penase LEO, Leo Pharmaceutical Products.

Anal.—Calc. for $C_{22}H_{30}N_4O_6S$: C, 55.22; H, 6.32; N, 11.71. Found: C, 55.02; H, 6.27; N, 11.87.

Synthesis of (6S)-6-[(Hexahydro-1H-azepin-1-yl)methyleneamino]penicillanic Acid Hydrochloride Hydrate—A solution of the above-mentioned benzyl ester (1.4 g, 2.9 mmoles) in ethyl acetate (25 ml) was successively extracted with sodium hydrogen carbonate (0.85 g) in water (25 ml) and water (25 ml). After drying, the ethyl acetate was removed *in vacuo*; the residue (1.3 g), dissolved in water (25 ml) and 4 *N* HCl (0.73 ml, 2.9 mmoles), was extracted with ether (25 ml) and hydrogenated over 10% palladium-on-carbon (1 g) at slightly elevated pressure.

The catalyst was filtered off, and the filtrate was extracted with ether (15 ml) and freeze dried. The product (0.8 g) was crystallized from isopropanol–isopropyl ether and 96% ethanol–ether to yield 0.45 g (43%) of the title compound, mp 170–173°, shown to be identical with the substance isolated from alkaline degradation of I by TLC, PMR, and IR.

(6*R*)-6-Formamidopenicillanic Acid (III)—This compound was synthesized from acetic formic anhydride and trimethylsilyl 6-aminopenicillanate and isolated as the sodium salt (13). It was recrystallized from formamide–acetone, mp 235–240°; $[\alpha]_D^{20}$ +272° (H_2O); PMR (D_2O, sodium 3-trimethylsilylpropanoate): 1.58 (s, 2αCH_3), 1.67 (s, 2βCH_3), 4.32 (s, 3H), 5.67 (m, 5H and 6H), and 8.23 (s, OCH); IR (0.3% KBr): 1782, 1672, 1599, 1490, 1388, 1312, 1125, and 778.

(6*S*)-6-Formamidopenicillanic Acid (IV)—*Benzyl (6S)-6-Formamidopenicillanate*—A mixture of formic acid (1.1 ml, 28.9 mmoles), triethylamine (4.0 ml, 28.9 mmoles), and acetyl chloride (2.1 ml, 28.9 mmoles) in tetrahydrofuran (130 ml) was stirred for 15 min at −70° and subsequently added to a solution of benzyl (6*S*)-6-aminopenicillanate (12) (9.1 g, 29.7 mmoles) in tetrahydrofuran (165 ml) at −70°. The reaction mixture was stirred for 3 hr at room temperature and filtered. The filtrate was evaporated *in vacuo*; the oily residue (15.6 g) was taken up in a mixture of ether (130 ml) and ethyl acetate (25 ml) and successively extracted with water (35 ml), dilute hydrochloric acid (35 ml, pH 2.5), and water (2 × 35 ml).

The organic phase was dried and evaporated *in vacuo*. Crystallization of the residue (11.5 g) from ethyl acetate–petroleum ether yielded 7.0 g (71%) of the pure compound, mp 107–108°; $[\alpha]_D^{20}$ +209° (96% C_2H_5OH); PMR ($CDCl_3$, tetramethylsilane): 1.38 (s, 2αCH_3), 1.57 (s, 2βCH_3), 4.53 (s, 3H), 5.0–5.4 (m, 5H and 6H), 5.18 (m, O$CH_2C_6H_5$), 7.10 (d, J = 8 Hz, NH), 7.37 (s, C_6H_5), and 8.18 (s, CHO); IR (0.3% KBr): 1785, 1738, 1655, 1525, 1450, 1382, 1212, 1186, 1004, 992, 742, 735, 695, and 578.

Anal.—Calc. for $C_{16}H_{18}N_2O_4S$: C, 57.47; H, 5.43; N, 8.38. Found: C, 57.56; H, 5.61; N, 8.36.

Potassium (6S)-6-Formamidopenicillanate—A mixture of the above-mentioned benzyl ester (1.0 g, 3 mmoles) in ethyl acetate (15 ml) and potassium hydrogen carbonate (0.9 g, 3 mmoles) in water (25 ml) was hydrogenated over 10% palladium-on-carbon (2.5 g) at slightly elevated

pressure. After filtration, the aqueous phase was separated, extracted with ether (25 ml), and freeze dried. The greyish product (1.2 g) was extracted with ethanol (25 ml) and filtered. The filtrate was evaporated *in vacuo* to leave a solid residue (0.8 g), which was triturated with isopropanol (10 ml). A 0.7-g (83%) yield of potassium (6*S*)-6-formamidopenicillanate as an amorphous powder was obtained; PMR (D_2O, sodium 3-trimethylsilylpropanoate): 1.52 (s, $2\alpha CH_3$), 1.60 (s, $2\beta CH_3$), 4.33 (s, 3H), 4.93 (d, J = 1.8 Hz, 6H), 5.34 (d, J = 1.8 Hz, 5H), and 8.18 (s, CHO); IR (0.3% KBr): 1750, 1660, 1600, and 1525.

(1′*R*,2*R*,4*S*)-5,5-Dimethyl-2-[1′-((hexahydro- 1*H* -azepin-1-yl)-methyleneamino)methyl]thiazolidine-1′,4-dicarboxylic Acid[7] (V)—A mixture of I (5.0 g, 15.4 mmoles) and 1,500,000 units of β-lactamase[6] in water (50 ml) at room temperature was kept at a constant pH (7.0) by adding 1 *N* NaOH. When no more sodium hydroxide was consumed, the solution was freeze dried to yield 5.7 g (100%) of the crude sodium salt of V.

To a solution of this salt (3.7 g, 10 mmoles) in water (30 ml) was added 2-naphthalenesulfonic acid trihydrate (6.6 g, 25 mmoles) in water (70 ml) at room temperature. The precipitate (5.5 g) was recrystallized from acetone (25 ml) and water (5 ml) by addition of ether (50 ml) and from methanol–ether to yield the pure 2-naphthalenesulfonate hydrate of V (1.6 g, 28%), mp 132–133°; $[\alpha]_D^{20}$ +30° (CH_3OH); PMR (CD_3OD, tetramethylsilane): 1.28 (s, $5\alpha CH_3$), 1.60 (s, $5\beta CH_3$), 1.5–2.1 [m, $(CH_2)_4$], 3.72 (s, 4H), 3.3–3.8 (m, CH_2NCH_2), 4.27 (d, J = 7 Hz, 1′H), 5.14 (d, J = 7 Hz, 2H), 7.4–8.3 (m, $C_{10}H_7$), and 8.38 (bs, NCH=N); IR (0.3% KBr): 1730, 1682, and 1620.

Anal.—Calc. for $C_{25}H_{35}N_3O_8S_2$: C, 52.71; H, 6.19; N, 7.37; H_2O, 3.16. Found: C, 52.62; H, 6.24; N, 7.18; H_2O, 3.78.

(2*R*,4*S*)-5,5-Dimethyl-2-[(hexahydro- 1*H* -azepin-1-yl)methyleneaminomethyl]thiazolidine-4-carboxylic Acid[8] (VII)—A suspension of 2-aminomethyl-5,5-dimethylthiazolidine-4-carboxylic acid dihydrochloride dihydrate (16) (30 g, 100 mmoles) in chloroform (800 ml) was stirred for 0.5 hr at room temperature. Triethylamine (28 ml, 200 mmoles) was added. After stirring for 2.5 hr, the precipitate (21.5 g) was filtered off and washed with chloroform (4 × 100 ml) and ether (2 × 100 ml).

To a stirred suspension of this crude zwitterion (19 g, 100 mmoles) in methanol (1000 ml) was added hexamethyleneiminecarboxaldehyde dimethyl acetal (18.2 ml, 100 mmoles) at room temperature. After 2 hr, the solution was filtered and evaporated *in vacuo*. Addition of water (7 ml) followed by acetone (100 ml) to the solid residue yielded 17.6 g of crude VII, mp 153–155°. Treatment of this product in methanol (240 ml)–ether (240 ml) with hydrogen chloride in isopropanol afforded the hydrochloride of VII, mp 183–184°.

Recrystallization from methanol–ether yielded 7.1 g (24%) of the pure VII hydrochloride, mp 186.5–187°; $[\alpha]_D^{20}$ +150° (H_2O); PMR (D_2O, sodium 3-trimethylsilylpropanoate): 1.45 (s, $5\alpha CH_3$), 1.72 (s, $5\beta CH_3$), 1.4–2.1 [m, $(CH_2)_4$], 3.4–3.8 (m, CH_2NCH_2), 3.87 (m, 1′H), 4.07 (s, 4H), 5.12 (t, J = 6 Hz, 2H), and 8.03 (s, NCH=N); IR (0.3% KBr): 1685, 1600, 1460, 1128, and 787.

Anal.—Calc. for $C_{14}H_{26}ClN_3O_2S$: C, 50.05; H, 7.80; N, 12.51. Found: C, 50.12; H, 7.72; N, 12.70.

(1′*R*,2*R*,4*S*)-5,5-Dimethyl-2-(1′-formamidomethyl)thiazolidine-1′,4-dicarboxylic Acid[9] (IX)—A solution of the III sodium salt (2.6 g, 10 mmoles) in 50 ml of water was treated at room temperature with 500,000 units of β-lactamase[6]. The pH was kept at 7.0 by adding 1 *N* NaOH (10 ml). After freeze drying, the IX disodium salt was obtained in 100% yield (3.0 g); PMR: (D_2O, sodium 3-trimethylsilylpropanoate): 1.27 (s, $5\alpha CH_3$), 1.61 (s, $5\beta CH_3$), 3.42 (s, 4H), 5.03 (d, J = 7 Hz, 2H), 4.32 (m, 1H), and 8.13 (bs, OCH); IR (0.3% KBr): 1665, 1595, and 1398.

(2*R*,4*S*)-5,5-Dimethyl -2- formamidomethylthiazolidine-4-carboxylic Acid[10] (XI)—A mixture of 2-aminomethyl-5,5-dimethylthiazolidine-4-carboxylic acid dihydrochloride dihydrate (16) (9.0 g, 30 mmoles), triethylamine (8.4 ml, 60 mmoles), and tetrahydrofuran (300 ml) was stirred for 3 hr at room temperature and subsequently cooled to −70°.

In another flask, formic acid (1.16 ml, 30.6 mmoles), acetyl chloride (2.18 ml, 30.6 mmoles), and triethylamine (4.26 ml, 30.6 mmoles) were stirred in tetrahydrofuran (120 ml) at −70° for 10 min. While stirring, the resulting suspension was immediately added to the first mixture. The temperature was raised to 20–25° during 1.25 hr. After filtration and washing with tetrahydrofuran, the filtrate was evaporated *in vacuo*.

[7] Previously named BB 511 (14, 15).
[8] Previously named FL 1115 (14).
[9] Previously named BB 517 (14, 15).
[10] Previously named FL 1114 (14).

Table II—R_f Values of Mecillinam and Reference Compounds

Compound	R_f Values System A	System B	System C	System D	System E
I	0.05	0.25	0.60	0.05	0.45
II	0.05	0.30	0.65	0.05	0.45
III	0.50	0.70	0.75	0.50	0.55
IV	0.40	0.65	0.70	0.35	0.50
V	0.00	0.10	0.45	0.00	0.35
VI	0.00	0.15	0.25	0.00	—
VII	0.00	0.15	0.50	0.05	0.50
VIII	0.00	0.15	0.45	0.05	0.45
IX	0.05	0.10	0.20	0.00	0.15
X	0.05	0.10	0.15	0.00	0.15
XI	0.20	0.25	0.35	0.15	0.45
XII	0.20	0.25	0.35	0.15	0.45
XIII	0.45	0.50	0.65	0.45	0.65
⊂NH[a]	0.10	0.15	0.45	0.20	0.50
6-Aminopenicillanic acid	0.15	0.20	0.40	0.25	—
Penicic acid	0.05	0.05	0.20	0.05	0.15
Penicillamine	—	—	0.45	0.10	0.55

[a] Only visible with 4,4′-tetramethyldiaminodiphenylmethane spray reagent (11).

The oily residue (8.6 g) was crystallized from methanol (40 ml) to yield 2.6 g (40%) of XI, mp 160.5–161°; $[\alpha]_D^{20}$ +169° (H_2O); PMR [$(CD_3)_2SO$, tetramethylsilane]: 1.21 (s, $5\alpha CH_3$), 1.57 (s, $5\beta CH_3$), 3.17 (m, 1′H), 4.65 (m, 2H), 3.58 (s, 4H), 7.5 (bs, 3NH and COOH), and 8.02 (bs, OCH); IR (0.3% KBr): 1680, 1635, 1555, 1135, 1060, 1045, 780, and 595.

Anal.—Calc. for $C_8H_{14}N_2O_3S$: C, 44.02; H, 6.46; N, 12.83. Found: C, 44.27; H, 6.43; N, 12.71.

(1′*R*,2*R*,4*S*)-5,5-Dimethyl-2-(1′-formamido- 1′ -*N*,*N*-hexamethylenecarbamoylmethyl)thiazolidine-4-carboxylic Acid[11] (XIII) —*Isolation from Alkaline Degradation of I*—A I solution (10 g, 31 mmoles) in water (100 ml) was adjusted to pH 10 and kept for 4 hr at 37° and constant pH. After adjustment to pH 3, the solution was extracted with chloroform (3 × 100 ml). The combined organic phases were dried and evaporated *in vacuo*. For chromatography, 0.5 g of the residue (1.8 g) was dissolved in chloroform (2 ml), applied to a Sephadex LH 20 column (65 × 2.5 cm), and eluted with chloroform (2 ml/min). The separation was followed by TLC using System B (*Assay Methods* and Table II).

The combined fractions containing XIII were evaporated *in vacuo* to leave an oil (0.29 g); PMR ($CDCl_3$, tetramethylsilane): 1.30 (s, $5\alpha CH_3$), 1.67 (s, $5\beta CH_3$), 1.6–2.0 [s, $(CH_2)_4$], 3.4–3.8 (m, CH_2NCH_2), 3.78 (s, 4H), 4.8–5.5 (m, 2H and 1′H), 7.02 (bs, 3H and COOH), 7.81 (bs, J = 9 Hz, CONH), and 8.33 (d, J = 1.2 Hz, HCO); ^{13}C-NMR ($CDCl_3$, tetramethylsilane): 59.8 (C-5), 67.0 (C-2), 52.3 (C-1′), 72.5 (C-4), 163.5 (HCO), 169.5 (NCO), and 171.7 (COOH); IR (5% $CHCl_3$): 1722, 1675, and 1625.

Synthesis—A solution of the III sodium salt (6.4 g, 25 mmoles) in water (75 ml) was stirred for 1.5 hr at room temperature with hexamethyleneimine (2.8 ml, 25 mmoles). After extraction with ether (25 ml), the aqueous phase was cooled (0–5°) and acidified (pH 3), followed by extraction with chloroform (5 × 100 ml). After drying, the chloroform was removed *in vacuo*. To a filtered solution of the residue (4 g, 12 mmoles) in acetone (80 ml) was added 1.4 ml (12 mmoles) of cyclohexylamine. Without isolation, the precipitated salt was brought into solution by the addition of water (12 ml).

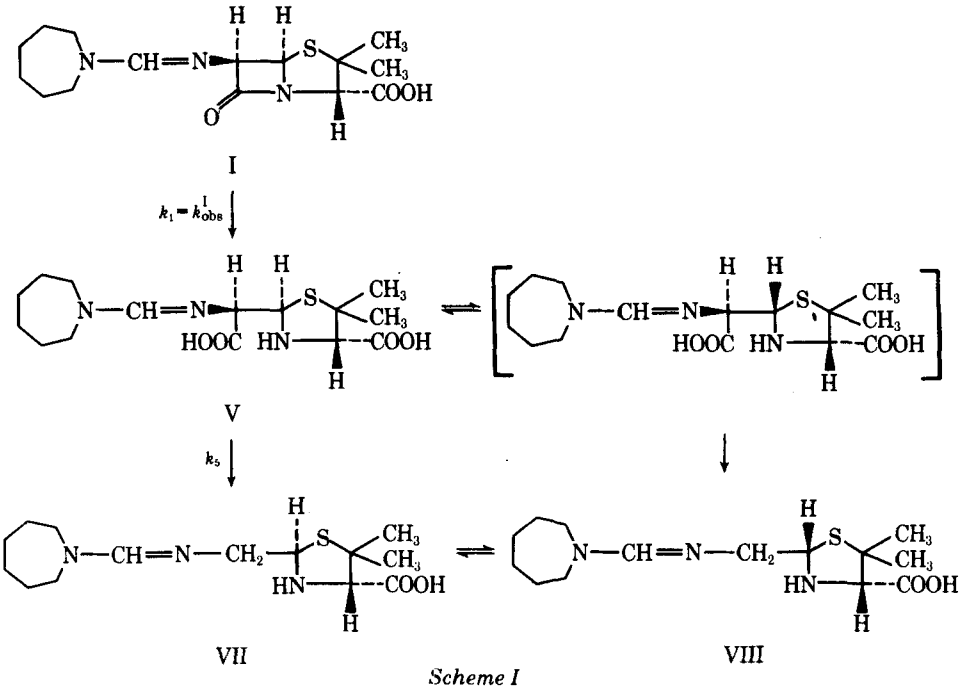
After filtration and addition of acetone (160 ml), 2.6 g (23% yield) of the XIII cyclohexylamine salt was obtained as a hemihydrate, mp 172–173°; $[\alpha]_D^{20}$ +96° (96% C_2H_5OH); PMR (D_2O, sodium 3-trimethylsilylpropanoate): 1.25 (s, $5\alpha CH_3$), 1.63 (s, $5\beta CH_3$), 1.2–2.3 [m, $(CH_2)_4$ and $(CH_2)_5$], 3.57 (s, 4H), 3.0–3.9 (m, CH_2NCH_2 and CH=N), 4.93 (m, 2H and 1′H), and 8.09 (s, CHO); IR (0.3% KBr): 1668, 1621, 1600, 1525, 1392, and 790.

Anal.—Calc. for $C_{21}H_{38}N_4O_4S \cdot 0.5H_2O$: C, 55.85; H, 8.71; N, 12.41; H_2O, 1.99. Found: C, 55.88; H, 8.67; N, 12.49; H_2O, 2.28.

Assay Methods—*Iodometric Method*—The residual concentration of intact β-lactam compound(s) was determined by an iodometric titration.

Spectrophotometric Method—The concentrations of mecillinam (I) and (6*S*)-mecillinam (II) were determined by a spectrophotometric measurement[12] at 330 nm of the imidazolone formed by reaction between I and glycine in aqueous solution (17).

[11] Previously named BB 514 (14).
[12] Beckman Acta C III.



Scheme I

TLC—The degradation products were identified and semiquantified by comparing samples of appropriate reference compounds in different solvent systems. The R_f values of the reference compounds are given in Table II.

Solvent systems were: A, ethyl acetate–water–acetic acid (75:10:15); B, ethyl acetate–water–formic acid (80:10:10); C, chloroform–ethanol (96%)–formic acid (50:30:20); D, chloroform–ethanol (96%)–acetic acid (80:20:30); and E, chloroform–acetone–acetic acid–water (40:30:30:10).

For a semiquantitative estimation of degradation products in Systems A and B, 10-μl samples from the reaction solution as well as 10-μl samples from a 10-fold aqueous dilution of the reaction solution were linearly applied directly to the silica gel plate[13]. The aqueous standard blends were applied beside the test samples. With Systems C–E, samples (1.0 ml) of the reaction solution were freeze dried and dissolved in methanol (1.0 ml). Half of this solution was diluted to 5.0 ml with methanol, and 10 μl of each methanolic solution was applied linearly to the plate together with methanolic samples of the appropriate reference compounds.

The plates were developed in each solvent system until the mobile phase had ascended 12.5 cm. The chromatograms were visualized by exposing the plate to iodine vapor, followed by spraying with a starch indicator (1% in water). For chromatograms developed in System D, the visualization was performed with 4,4′-tetramethyldiaminodiphenylmethane reagent (18).

NMR Methods—The NMR data[4] of the pure compounds were obtained from 10% (w/v) solutions. Kinetic studies were performed either on freeze-dried samples redissolved in deuterium oxide or on samples taken directly from experiments performed in deuterium oxide or water. Deuterated acid or base was used for pD adjustment in deuterium oxide. The overall concentration of the kinetic experiments was ~1%, and hexamethyldisiloxane served as the internal reference.

In Fourier transform NMR, deuterium from the deuterated solvents was used as the internal lock. In water, a coaxial inner tube[14] with deuterium oxide served as the external lock. The digital resolution was kept better than the spectrometer resolution, and from 24–200 (proton) up to 60,000 (carbon 13) scans were accumulated. Usually, a carbon–proton 5-mm dual probe or, in special cases, a 10-mm carbon probe was used.

All spectra were obtained with 30 or 90° pulses, using a sufficient repetition time to allow full relaxation.

Homo and hetero decoupling experiments were performed with standard 8K software and standard hardware. The water band was mostly eliminated through homogated decoupling.

Overhauser enhancement was obtained from degassed samples. During data manipulation, a light positive exponential multiplication was performed. Whenever necessary and possible, reference compounds were added to the reaction mixtures in realistic concentrations to support individual compound identification and to facilitate proton spectrum interpretation.

Kinetic Procedures—An accurately weighed amount of I was dissolved in preheated water (37°) to produce an initial concentration of $\sim 3 \times 10^{-2}$ M (1% w/v). Hydrochloric acid or sodium hydroxide was added to give the reaction solution the desired pH (all integers of the pH interval from 2 to 10), and the pH was maintained by a pH-stat[15]. Before and after an experiment, the pH meter was standardized with a standard buffer at the same temperature. No significant pH change was observed. The reactions were carried out at constant temperature (37 ± 0.1°).

If the half-life of I was more than 1 day, a closed titration vessel was used. In some experiments (at pH 2, 5, and 8), the ionic strength was adjusted to 0.5 by addition of sodium chloride. Samples from the reaction solutions were taken at appropriate intervals and analyzed. Aqueous solutions of II, 6-formamidopenicillanic acid (III), and mecillinam penicilloic acid (V)[16] (1% w/v) were subjected to the same kinetic procedure.

Table III—Acidic Hydrolysis of Mecillinam (1% w/v) at pH 2 and 37°

Hours	Product Distribution, mole %			
	I	V	VII	VIII
0.25	99	2	—	—
3	90	5–10	2	—
6	80	5–10	10	5
24	35	5–10	35	25
50	15	5–10	45	35

RESULTS[16]

The degradation of mecillinam (I) was followed until most of the drug was destroyed. The results obtained at pH 2, 6, and 10 will suffice to outline the influence of pH on hydrolysis rate and product distribution.

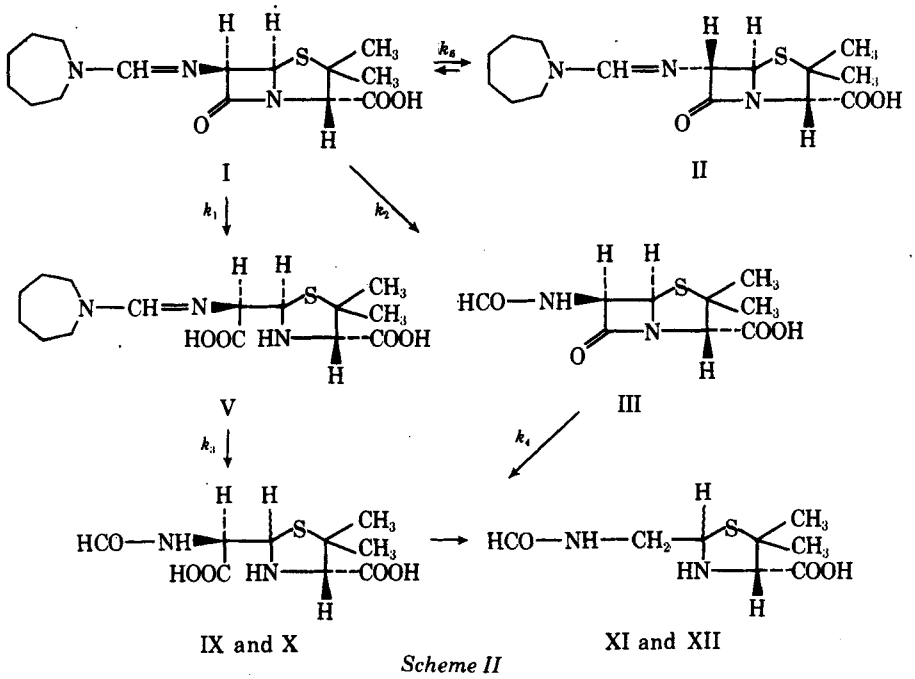
The structures of the degradation products with unchanged stereochemistry were deduced by comparing their R_f values with those of synthetic specimens of likely candidates in several systems and were

[13] Silica gel 60 F_{254} (Merck 5715).

[14] Type WGS-5 BL, Wilmad Glass Co.

[15] Radiometer model TTT 1 b, SBR 2, and magnetic valve type MNVIC or Radiometer RTS 622.

[16] Although not strictly correct, the nomenclature and numbering of penicillin are used for all degradation products including monocyclic compounds except in the *Experimental* part. When not otherwise indicated, the compounds have the natural penicillin configuration (3*S*, 5*R*, 6*R*).



Scheme II

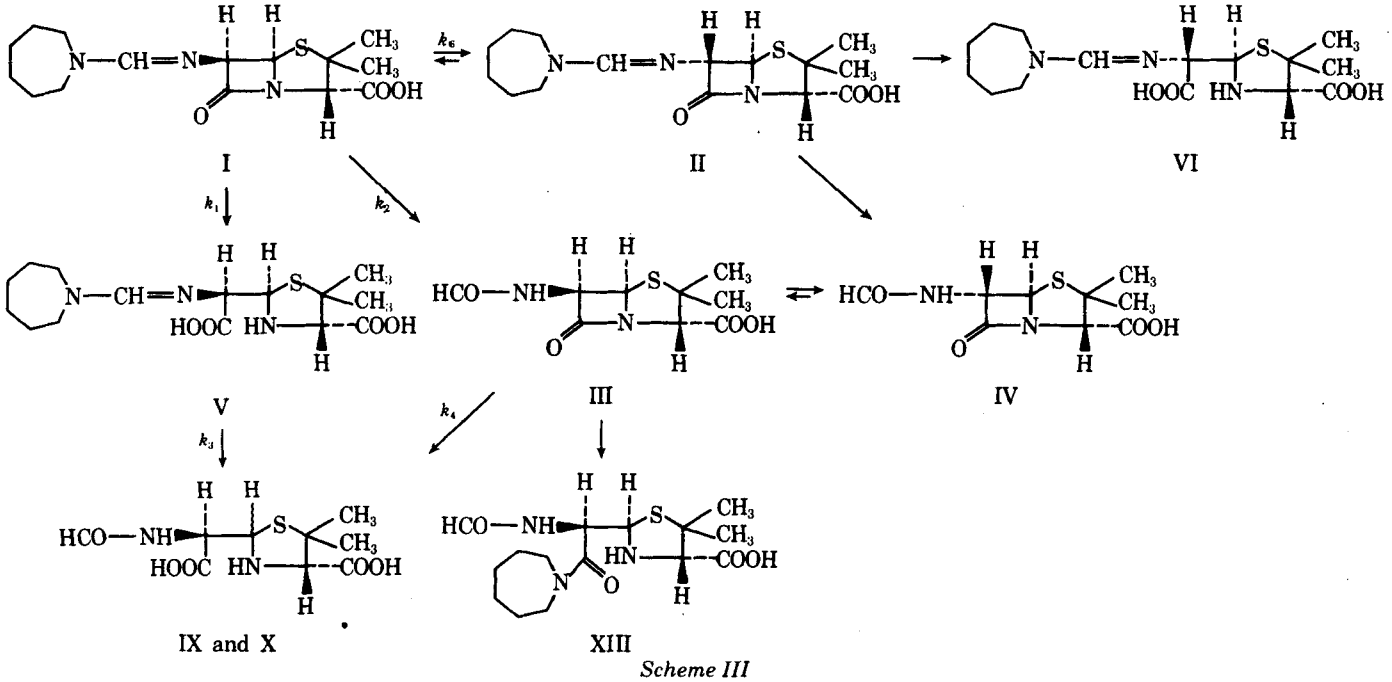
confirmed by NMR spectroscopy, except in the case of the penicilloic amide XIII (Table I). This compound, as well as (6*S*)-mecillinam (II), was initially isolated from reaction mixtures and identified. For the isolation of II, use was made of the resistanceof II toward β-lactamase (*cf.*, 11) to separate it from I.

The existence of other stereochemically abnormal degradation products, when suspected from NMR, was verified by equilibration experiments on the appropriate reference compounds with normal configuration. The structures of XIII, II, and (6*S*)-formamidopenicillanic acid (IV) were further confirmed by unambiguous syntheses. All were straightforward. The yields of degradation products were estimated by comparison with reference compounds by TLC and further supported by NMR.

In strong acid solution (pH 2, Table III and Scheme I), the β-lactam ring of I was opened rapidly to afford the penicilloic acid V in 5–10% yield. No further increase in the yield of this compound was noted during the next 50 hr. In the same period, an increasing amount of decarboxylated product (VII) was formed. After 50 hr, a 45% yield of VII was obtained. The other major product formed concurrently with VII, and in almost the same yield, was the (5*S*)-epimer VIII. The half-life of I at pH 2 was ~15 hr (k_{obs} = 0.039 hr^{-1}, Table IV). No compounds resulting from amidine bond hydrolysis were observed.

In weak acidic solution (pH 6) about its isoelectric point, I degraded more slowly, the half-life being ~100 hr (Tables IV and V and Scheme II). Amidine bond hydrolysis occurred during the formation of most of the observed products. In addition to hexamethyleneimine, the predominant products formed were 6-formamidopenicillanic acid (III) and compounds formally derived from it by hydrolysis of the β-lactam ring (IX and X) and subsequent decarboxylation (XI and XII). A 10% yield of II was noticed after 120 hr.

In strong basic solution (pH 10, Table VI and Scheme III), I was rapidly destroyed with a half-life of ~1 hr. Compounds undergoing amidine bond hydrolysis during the degradation formed a major part of the reaction mixture. Thus, the 6-epimeric formamidopenicillanic acids III and IV, the 5-epimeric penicilloic acids IX and X, and the penicilloic amide XIII were formed in a total yield of 50–60% during 2–3 hr, with XIII being of



Scheme III

Table IV—Measured Rate Constants [a] at 37°

pH	k^{I}_{obs}, hr^{-1}	k^{II}_{obs}, hr^{-1}	k_3, hr^{-1}	k_4, hr^{-1}
2	0.039	0.0026	—	—
6	0.0076	0.0016	0.22	0.0022
10	0.79	0.29	0.32	0.32

[a] Estimated error <20%.

Table V—Weak Acidic Hydrolysis of Mecillinam (1% w/v) at pH 6 and 37°

Hours	Product Distribution, mole % I	II	III	IX + X [a]	XI + XII	⊂NH
0.25	99	—	—	—	—	—
2	99	—	—	0.5	—	—
5	95	0.5	0.5	2	—	3
24	85	1	3	10 (1:1)	—	15
50	70	5	7	20 (1:1.2)	2	25
120	40	10	15	30 (1:1.5)	10	50

[a] Figures in parentheses are the IX:X ratios estimated by NMR.

secondary importance. A 55–65% yield of hexamethyleneimine was obtained during the same period.

At the same time, compounds with the preserved amidine bond (the major component II, V, and VI) were produced in a 30% yield. 6-Aminopenicillanic acid, penicic acid, and penicillamine were not observed.

As seen from Tables VII and VIII, the yield of XIII was dependent on the I concentration. Increasing this concentration from 0.1 to 10% increased the yield of XIII.

In addition, experiments performed at pH 2, 5, and 8, either neat or with added sodium chloride to obtain an ionic strength of 0.5, showed no influence of ionic strength on the degradation rate and product distribution.

DISCUSSION

Methods—Although TLC has infrequently been applied to the study of penicillin degradation, several solvent systems have been developed for the separation of the penicillins and their major degradation products (19). In the present study, special solvent systems had to be developed in consequence of the strongly polar compounds formed from mecillinam (I). No single system was discovered that could separate all degradation products. Only with several systems did TLC on silica gel satisfactorily separate products formed in at least a 0.5% yield.

Many solvent systems were evaluated to ensure that no principal products were overlooked and that no artifacts were interpreted as degradation products. This procedure was supported by NMR and semiquantitative measurements. As seen from Tables III and V–VIII, the yields of degradation products accounted for nearly all lost I.

Generally, these results were estimated to be accurate with a relative uncertainty of 20%, although the determinations of hexamethyleneimine in basic reaction resolutions were more uncertain because of the unavoidable evaporation of some amine during the freeze-drying process involved in the TLC and NMR determinations.

PMR spectra in deuterium oxide or water could be used to separate and quantitate all products containing a formyl or an amidine proton (Fig. 1). As expected, large pD, pH shifts (up to 0.5 ppm) were observed in the formyl and amidine 8-ppm region. The amidine protons were more sensitive to pD, pH variations than were the formyl protons (Table IX).

The best separation of the hydrolysis compounds was obtained in basic solutions where all amidines could be detected separately to yield satisfactory integrals. In the absence of overlapping lines, integrals could be determined better than ±5%. With partly overlapping lines, the estimated error was <±25%. By changing the pD during observations, useful integrals of nearly all of the hydrolysis products could be obtained. Destruction products formed in yields of <5% could not be determined because of further degradation during the necessary accumulation time.

Outside the 8-ppm region, it was possible to identify all compounds through characteristic absorptions. However, quantitation of the hydrolysis mixture using these absorptions was often less profitable due to overlapping spin patterns.

The NMR results were in excellent agreement with the TLC observations. In some cases, products (V and VIII–XII) that could only be quantitated uncertainly, or as a sum from TLC, could be determined separately.

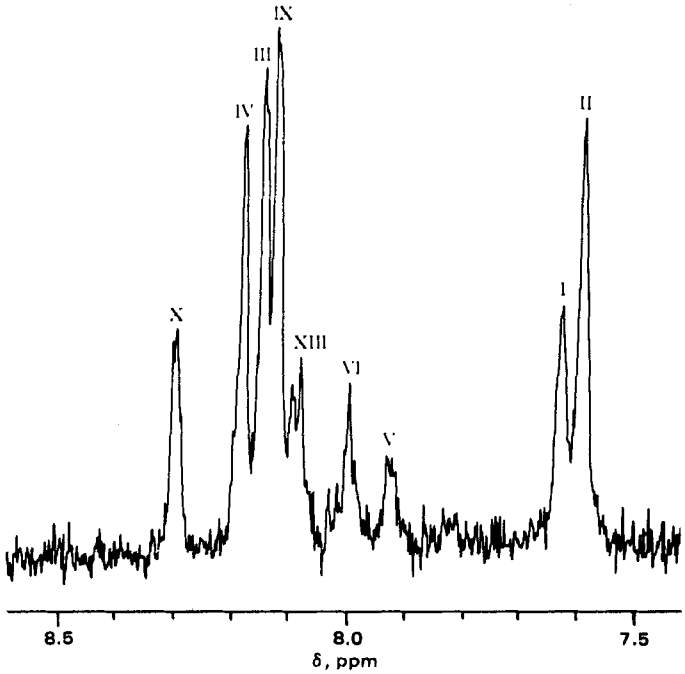


Figure 1—*PMR spectrum (99.6 MHz) of a freeze-dried sample from I hydrolysis (1% w/v, 3 hr, 37°, pH 10). Sample was redissolved in deuterium oxide and adjusted to a pH meter reading of 10.2; the 8-ppm region is shown. Conditions were: number of pulses, 64; flip angle, 30°; pulse repetition time, 15 sec; and digital resolution, 0.12 Hz.*

In addition to the TLC and NMR methods, intact I also has been determined by iodometric titration and a spectrophotometric method recently developed by Larsen and Bundgaard (17). Whereas the iodometric assay does not distinguish between different types of β-lactam-containing compounds, the spectrophotometric method is claimed to be highly specific for I and its pivaloyloxymethyl ester (pivmecillinam) since an intact β-lactam ring, as well as an amidine group at position 6, is necessary for the method. Under weak acidic to basic conditions, this method is invalidated due to the formation of II. The molar absorbance of II when subjected to this assay is only about 75% of the absorbance of I, presumably owing to slower reaction with the glycine reagent since the same 4-aminomethyleneimidazol-5(4*H*)-one derivative should be formed from both epimers. Therefore, this spectrophotometric assay cannot be used as a general specific method for quantitation of I under weak acidic to basic conditions.

Reaction Rate Constants—At constant pH and temperature, the observed rate for I degradation, obtained by measuring the remaining intact I, followed pseudo-first-order kinetics. The observed rate constants (k^{I}_{obs}) at pH 2, 6, and 10 are listed in Table IV. At pH 6, k^{I}_{obs} was the sum of three rate constants: k_1 (β-lactam opening), k_2 (hydrolysis of the amidine bond), and k_6 (6-epimerization). To calculate k_1, k_2, and k_6 at pH 6, the following differential equations were used:

$$\frac{d(\mathrm{I})}{dt} = -k^{I}_{obs}(\mathrm{I}) = (-k_1 - k_2 - k_6)(\mathrm{I}) \qquad \text{(Eq. 1)}$$

$$\frac{d(\mathrm{III})}{dt} = k_2(\mathrm{I}) - k_4(\mathrm{III}) \qquad \text{(Eq. 2)}$$

$$\frac{d(\mathrm{II})}{dt} = k_6(\mathrm{I}) - k^{II}_{obs}(\mathrm{II}) \qquad \text{(Eq. 3)}$$

where the observed rate constants for the degradation of II (k^{II}_{obs}) and III (k_4) were separately determined (Table IV).

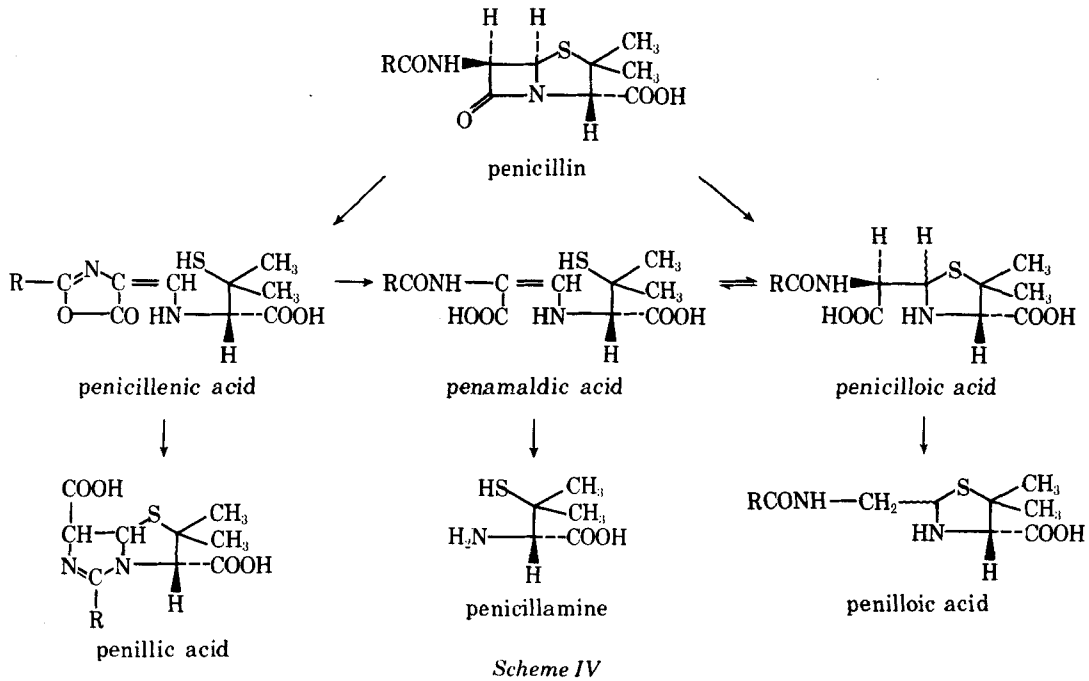
The solutions of the differential equations are:

$$k_2 = \frac{[\mathrm{III}][k_4 - k^{I}_{obs}]}{[\mathrm{I}]_0[e^{-k^{I}_{obs}t} - e^{-k_4 t}]} \qquad \text{(Eq. 4)}$$

$$k_6 = \frac{[\mathrm{II}][k^{II}_{obs} - k^{I}_{obs}]}{[\mathrm{I}]_0[e^{-k^{I}_{obs}t} - e^{-k^{II}_{obs}t}]} \qquad \text{(Eq. 5)}$$

$$k_1 = k^{I}_{obs} - k_2 - k_6 \qquad \text{(Eq. 6)}$$

Comparison of Penicillin and Mecillinam Hydrolysis—In addition



Scheme IV

to identifying the major hydrolytic degradation products of I, it was of interest to compare the influence of an amidine and an amide side chain on the hydrolytic patterns of their 6-aminopenicillanic acid derivatives. However, due to the complexity of penicillin transformations in aqueous solution (20), no completely satisfactory degradation schemes have been put forward for penicillin hydrolysis. Among the schemes found in the literature (2, 19, 21–29), only those of Blaha *et al.* (27) and Hartmann *et al.* (28) are exclusively based on accurate analyses. They followed the formation of a selection of known degradation products of penicillins G and V in aqueous solution as a function of pH, until most of the penicillins were destroyed, by high-pressure liquid chromatography (HPLC) and UV spectroscopy. Though slightly different, and despite the inherent incompleteness, their schemes with minor modifications are adopted in the discussion as representing the main features of penicillin degradation.

Acidic Solution—Scheme IV shows the degradation of penicillins in acidic solution. Nearly all of the primary degradation products of a penicillin retain the side chain either intact or in derived form (penicillenic and penillic acid). The only exception is penicillamine solely derived from the nucleus. The scheme differs from those of previous investigators (27, 28) mainly by the incorporation of the 5-epimerization of penicilloic acid. (5*S*)-Penicilloic acid was not included among their reference compounds. Depending on pH and temperature, at least some epimerization can be expected before decarboxylation to the 5-epimeric mixture of penilloic acids.

In I, the side chain is also preserved during acidic hydrolysis (Scheme I). Cleavage of the β-lactam ring initiates the formation of a series of 5-epimeric compounds closely corresponding to those found in the penicillin case with an intact side chain and a thiazolidine ring. No compounds of the penillic or penicillenic acid types are formed, as shown by the absence

Table VI—Basic Hydrolysis of Mecillinam (1% w/v) at pH 10 and 37°

Hours	Product Distribution, mole %								
	I	II	III	IV	V	VI	IX + X[a]	XIII	⊂NH
0.25	95	1	3	—	1	—	1	—	3
1	50	20	10	4	5	—	10	0.5	30
2	20	20	20	7	10	1	20 (5.5:1)	1	55
3	10	20	20	15	5	5	25 (4.5:1)	2	65
4	5	20	15	15	1	5	35 (2.5:1)	5	75

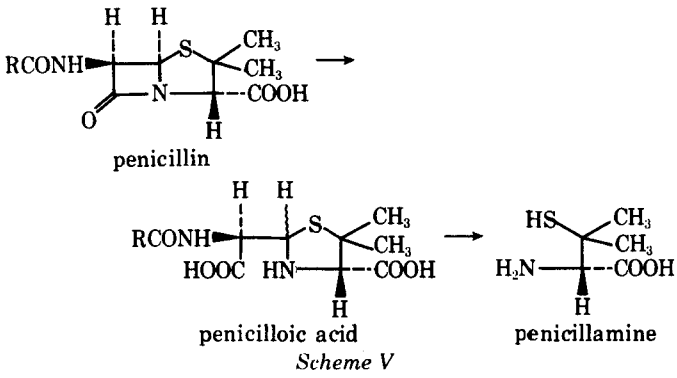
[a] Figures in parentheses are the IX:X ratios estimated by NMR.

Table VII—Basic Hydrolysis of Mecillinam (0.1% w/v) at pH 10 and 37°

Hours	Product Distribution, mole %								
	I	II	III	IV	V	VI	IX + X	XIII	⊂NH
0.25	95	1	3	—	1	—	1	—	3
1	50	20	10	4	5	0.5	10	0.5	30
2	20	20	20	7	10	1	20	1	55
3	10	20	20	15	10	2	25	2	65
4	5	20	15	15	5	2	35	5	75

Table VIII—Basic Hydrolysis of Mecillinam (10% w/v) at pH 10 and 37°

Hours	Product Distribution, mole %								
	I	II	III	IV	V	VI	IX + X	XIII	⊂NH
0.25	95	2	5	—	—	—	—	—	5
1	50	20	10	5	1	0.5	2	1	30
2	20	25	20	15	2	1	15	4	45
3	10	20	15	20	2	1	20	8	65
4	5	20	10	20	2	2	25	20	65



Scheme V

of hexamethyleneimine and a UV maximum higher than 260 nm when the hydrolysis was followed through 96 hr at pH 4 and 37°.

The observed (5*S*)-penilloic acid (VIII) is believed to result from 5-epimerization of the major degradation product (VII) rather than from a decarboxylation of the unobserved (5*S*)-penicilloic acid. This view was supported by epimerization experiments (NMR) of V and VII since V underwent rapid decarboxylation (k_5 = 0.4 hr^{-1}) and VII, under the same conditions (1 hr, pH 2, 37°), was converted to a 5-epimeric mixture of VII (60%) and VIII (40%).

During similar epimerization experiments in deuterium oxide, little, if any, deuterium incorporation at position 3, 5, or 6 took place. These findings indicate that 5-epimerization involving intermediates of the penamaldic acid type, as postulated for penicilloic acids of true penicillins, must be unimportant.

Weakly Acidic or Neutral Solution—Fewer degradation products, as well as an increased stability of penicillins, was observed under these conditions. The primary Scheme IV products still arose, but little, if any, penilloic acid or penicillamine was produced and penillic acid was not formed.

In contrast to penicillins, and in spite of the increased stability about the isoelectric point (5.8), additional degradation routes were observed for I (Scheme II). As in the acidic hydrolysis, the major part was degraded through the penicilloic acid V route (k_1 calculated to 0.005 hr^{-1}) with an intact side chain. The very low yield (<1%) of V underemphasized the importance of this route due to the subsequent rapid hydrolysis of the amidine bond of V (k_3 = 0.22 hr^{-1}, pH 6, 37°, Table IV) to yield IX, X, and hexamethyleneimine.

A second, less important degradation route leading to IX through III ("hydrogenpenicillin") involves the same hydrolytic reactions but in reversed order. The relatively low rate constant for the amidine bond hydrolysis (k_2 calculated to 0.002 hr^{-1}) as compared with the hundred times greater k_3 points to an intramolecularly assisted hydrolysis of the α-amidinocarboxylic acid V. A similar proposal was recently put forward by other investigators (30) who suggested the formation of a 4-(thiazolidin-2-yl)-oxazol-5-one intermediate with simultaneous expulsion of hexamethyleneimine.

The key degradation product (IX) was further transformed to its 5-epimeric product (X); in acidic solution, both compounds were decarboxylated to yield an epimeric mixture of the penilloic acids XI and XII. The mecillinam penicilloic acid V, on the other hand, was not decarboxylated because of the rapid competitive amidine bond hydrolysis to IX. This proposed degradation route for V was supported by the stability of the unobserved penilloic acid VII, which was stable for more than 24 hr at pH 6.

A third degradation route, unprecedented in the penicillin series, was a commencing epimerization at the 6-position. This epimerization was rather slow at pH 6 (k_6 calculated to 0.001 hr^{-1}), as was the further degradation of (6*S*)-mecillinam (II) when compared to I. No further 6-epimerized products were observed.

Basic Solution—At higher pH, the penicillin degradation (Scheme V) was even more simplified. The penicilloic acid was the only primary degradation product, presumably as a 5-epimeric mixture (31).

In basic solution, the primary steps of the I degradation (Scheme III) were identical to those in weakly acidic and neutral solutions (Scheme II). In contrast, the secondary steps showed increased complexity.

Because of the intricate degradation scheme, it was not possible from the results in Table VI to calculate k_1 (β-lactam opening), k_2 (amidine hydrolysis), and k_6 (6-epimerization) (Scheme III) with reasonable certainty. Nevertheless, some qualitative considerations can be given by combining the results in Tables IV and VI.

A drastic increase in k_1 as compared to k_3 occurred. This result follows from the observed degradation rate constant for I (Table IV) and the separately determined, almost unchanged, k_3 together with the detection of considerable amounts of V.

Furthermore, k_2 was the same order of magnitude or even greater than k_4 since the 150-fold increase in k_4 at pH 10 as compared to pH 6 was not reflected in a decrease in the III concentration, despite the two additional degradation routes for this compound. The increase of k_2 with increasing pH is a logical consequence of the lower stability of amidines in basic solutions (6). The small pH dependence of k_3 for the hydrolysis of the α-amidinocarboxylic acid (V) may reflect a special hydrolysis mechanism of this type of amidine compound.

As a third important degradation route, the 6-epimerization yielding II can be observed. It was not possible to determine the relative importance of the routes I → V, I → III, and I → II. However, the results in Tables IV and VI suggest that k_1, k_2, and k_6 are in the same order of magnitude.

In a secondary degradation step, the II hydrolysis afforded substantial amounts of (6*S*)-6-formamidopenicillanic acid (IV). This compound also can be produced from III, as proved in separate experiments. This is the first reported example of a 6-epimerization in aqueous solution of a true penicillin. Compound II can further degrade to a (6*S*)-penicilloic acid (VI), and small amounts of VI were observed. It could be expected that IV is degraded in a similar way as III to yield 6- and 6,5-epimeric products. No such compounds were observed. The only 5-epimeric compound detected was X.

A minor product from a secondary step is the penicilloic amide XIII, which has no counterpart in the penicillin degradation schemes. Compound XIII is not a primary degradation product of I, resulting from intramolecular nucleophilic attack of the amidine group on the β-lactam moiety. Although this mechanism is favored by Larsen and Bundgaard (7), the influence of the I concentration on the yield of XIII (Tables VII and VIII) clearly showed that XIII is formed by an intermolecular reaction, presumably between III and hexamethyleneimine or, alternatively, by an initial bimolecular reaction between two molecules of I, or between I and hexamethyleneimine, followed by hydrolysis of the amidine bond. The first mechanism is preferred since XIII does not appear until substantial amounts of III have been formed (Table VI). Besides, addition of 1 equivalent of hexamethyleneimine to a 1% aqueous solution of I at pH 10 and 37° was without influence on the I half-life, although the yield of XIII was increased at the expense of III. Furthermore, the half-life of III under similar conditions was decreased 50% when an equimolar amount of hexamethyleneimine was added.

6-Epimerization—At basic pH, the sum of residual I and produced III derived from TLC was smaller than the amount of β-lactam-containing compounds determined from iodometric titration. In the PMR spectra of the hydrolysis mixture at pH 10, a freeze-dried sample redissolved in deuterium oxide and adjusted to a pH reading of 10.4 showed two singlets (4.27 and 4.24 ppm) and two doublets [5.20 ppm (J = 1.1 Hz) and 5.32 ppm (J = 1.7 Hz)]. This finding suggests the existence of at least two more β-lactam-containing compounds. The small coupling constants indicate that in these compounds the 5- and 6-protons are *trans*. Further analysis of the PMR spectra supports this theory.

No epimerization at position 6 in intact penicillins subjected to base-catalyzed hydrolysis has been reported, whereas 6-epimerization has been described for other 6-aminopenicillanic acid derivatives [hetacillin (32) and 6-trimethylammonium penicillanic acid (33)] in aqueous solution.

Table IX—PMR Data of Mecillinam Degradation Products, δ-ppm Region, as a Function of pH [a]

Compound	pH Meter Reading		
	2	5.6	10.2
I	8.000	7.995	7.631
II	8.019	8.035	7.580
III	8.149	8.145	8.144
IV	8.159	8.137	8.177
V	8.035	7.943	7.930
VII	7.985	7.897	7.870
VIII	8.021	7.992	7.940
IX	8.188	8.137	8.122
X	8.243	8.291	8.298
XI	8.162	8.064	8.050
XII	—	8.139	8.137
XIII	8.189	8.090	8.078

[a] Spectra were taken in deuterium oxide. All data were obtained by using hexamethyldisiloxane as the reference but were converted to trimethylsilylpropanoate as the 0.000-ppm delta scale. First-order approximations were used. Uncertainty on the parts per million reading was ±0.005 ppm. Probe temperature was 28°.

In view of these findings, the two unknown compounds were expected to be II and IV, respectively. Spectroscopic analysis of II isolated from the hydrolysis mixture proved the expected structure. Finally, II and IV synthesized in an unambiguous way were identical to the two β-lactam-containing hydrolysis products. In agreement with this finding, basic hydrolysis of I performed in deuterium oxide showed deuterium incorporation at position 6 but no incorporation at position 5.

In hydrolysis experiments at pH 10 (37°), II equilibrated to a mixture of I and II. After 3 hr ($t^{II}_{1/2}$ = 2.4 hr), 2–5 mole % of I appeared. This result was in contrast to hetacillin, where no (6*R*)-hetacillin could be detected from (6*S*)-hetacillin (33, 34).

The formation of IV took place mainly from II and, unexpectedly, to a smaller degree from III (see the preceding section). Similarly to II, IV epimerized to III (pH 10, 37°), giving approximately 1 mole % of III after 4 hr ($t^{IV}_{1/2}$ = 9.5 hr). No epimerization of a (6*S*)- (or 6*R*-) penicillin was reported previously.

Among the β-lactam-opened thiazolidines, the only 6-epimeric compound observed was the penicilloic acid VI.

5-Epimerization—No 5-epimerization of penicillins has been reported in aqueous solution. In contrast, thiazolidines are known to be in equilibrium with their thiol precursors (35). 5-Epimerization of the β-lactam-opened compounds, *e.g.*, the penicilloic acids, is well documented (21, 31, 36, 37).

In the present work, only the β-lactam-opened compounds were found to undergo 5-epimerization in acidic (pH 2), neutral (pH 7), and basic (pH 10) solutions. Rate constants and equilibrium proportions for the different compounds were found to depend on the side chain at position 5[17].

In all observable cases[18], epimerization experiments performed in deuterium oxide yielded compounds that were not deuterated at positions 3, 5, and 6. These findings are consistent with observations for 6-aminopenicillanic acid (31) where no deuterium incorporation during base-catalyzed hydrolysis could be detected. Several mechanisms have been proposed for the 5-epimerization, either through different enamine- (21, 28, 37) or azomethine- (31) type intermediates. From these results, C-6 cannot be involved in the epimerization process, excluding an enamine-type intermediate. Therefore, it must be concluded that at least the thiazolidines derived from I 5-epimerize like 6-aminopenicillanic acid (31) through intermediates of the azomethine type.

3-Epimerization—No sign of 3-epimerization of penicillins (38) or of I or its degradation products was detected.

(5S,6S)-Compounds—In the present work, no β-lactam-containing compounds with both 5- and 6-epimerization were observed.

From the degradation of II and IV and from the work of Busson *et al.* (36), it would be expected that some (5*S*,6*S*)-compounds must be present at least in basic solutions. No such compounds were observed. Due to the relatively greater stability of II and IV, the amount of (5*S*,6*S*)-compounds is believed to be of minor importance and they accordingly are not included in Schemes II and III.

SUMMARY

Aqueous solutions of mecillinam (I), unlike the penicillins, showed an increased complexity of major degradation routes and compounds with increasing pH. Some degradation reactions are known from penicillin chemistry but others are not, resulting in new types of degradation products at pH ≥ 6. Conversely, several of the structures from acidic degradation of penicillins were not reproduced at pH < 6.

In acidic solution, only compounds closely corresponding to penicillin degradation products with an intact side chain and a thiazolidine ring were formed, *i.e.*, penicilloic acid V[16] (Table I) and 5-epimeric penilloic acids VII and VIII of I.

In weakly acidic or neutral solutions, hydrolysis of the I and V amidine bonds yielded the key degradation product (6*R*)-6-formamidopenicillanic acid (III) and its 5-epimeric penicilloic (IX and X) and penilloic (XI and XII) acids together with hexamethyleneimine. A reversible 6-epimerization of I to yield (6*S*)-mecillinam (II) constituted a third, less important degradation route of I.

In basic solution, the same products, except XI and XII, were formed. Further II degradation led to the penicilloic acid VI. In addition to β-lactam opening, III degraded *via* a reversible 6-epimerization to (6*S*)-6-formamidopenicillanic acid (IV) and by reaction with hexamethyleneimine to the penicilloic amide XIII.

[17] Unpublished data.

[18] In some experiments, the position-6 protons were partly or completely hidden by the water band, and quantitative observations could not be done even under homogated decoupling experiments.

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ACKNOWLEDGMENTS

The authors gratefully acknowledge the skillful technical assistance of Mrs. Marie Pedersen, Mrs. Lis Finderup Johansen, and Mr. Gerhard Andersen and the helpful discussions about reaction kinetics with Dr. E. Ahlmann.