On the Mechanism of Rearrangement of 6-Halopenicilloates to 2,3-Dihydro-1,4-thiazines

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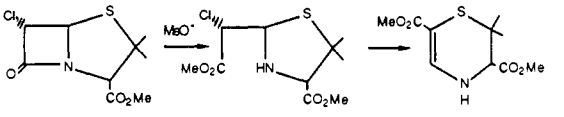
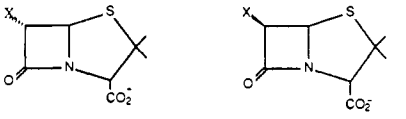
Abstract: The kinetics and mechanism of the rearrangement of 6-halopenicilloates to 2,3-dihydro-1,4-thiazines have been studied. First, the detailed sequence of the epimerization, hydrolysis, and rearrangement reactions of 6α- and 6β-chloropenicillanate was elucidated. This allowed the rate constants for rearrangement of 6α- and 6β-chloropenicilloate, 6α-bromopenicilloate, and 6α-iodopenicillanate to be confidently assigned. The rate constants for rearrangement of 6β-bromo- and 6β-iodopenicilloate were too large to be determined (probably greater than 100 s^{-1}). The rates of rearrangement of the bromo and iodo compounds were much greater than those of the chloro compounds, and those of the β-compounds were much greater than those of the α-compounds. The rates were pH-independent between pH 7.5 and 13.8, and increased with increase in ionic strength and increase in dielectric constant of the solution. The solvent deuterium isotope effect on the rearrangement of 6α-chloropenicilloate is 1.14 ± 0.02, and the isotope effect brought about by deuterium substitution at C-6 of this compound is 1.03 ± 0.02. The analogous rearrangement of the methyl esters of 6α-chloropenicilloate and 6α-bromopenicilloate and the amide of the chloro compound were also studied. Although these derivatives behaved similarly to the penicilloates at low pH (7–9), other reactions involving transient enamine formation were observed in hydroxide ion solutions. Nonetheless, there was no unambiguous evidence of a different rearrangement mechanism at high pH. The results of these experiments were used to evaluate a variety of possible mechanisms for the rearrangement reaction. It was concluded that the mechanism best fitting the facts involves direct intramolecular nucleophilic attack by the thiazolidine sulfur atom on the alkyl halide, yielding a bicyclic episulfonium ion intermediate, which collapses to product through intramolecular participation (perhaps concerted to some extent with the nucleophilic displacement) by the lone pair on the thiazolidine nitrogen atom.

The rearrangement to a dihydrothiazine following the nucleophilic cleavage of the β-lactam ring of a derivative of a 6-halopenicillanic acid (**1**) was first described by McMillan and Stoodley in 1966.[1] They showed that on methanolysis the methyl ester of 6α-chloropenicillanic acid was converted into 3,6-dicarbomethoxy-2,2-dimethyl-2,3-dihydro-1,4-thiazine by the reaction sequence of Scheme I. Some time later, interest in this rearrangement was rekindled through the discovery that certain 6β-halopenicillanic acids were potent inhibitors of certain β-lactamases[2,3] and that the mechanism of the inhibition involved this reaction.[4,5]

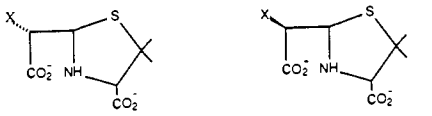
Although there have been a variety of mechanisms proposed for the rearrangement (see Discussion), very little evidence has been adduced for any one of them. The work described in this paper was undertaken to provide a better experimental framework for the consideration of these mechanisms. Knowledge of the mechanism should permit better understanding of the relative effectiveness of the 6-halopenicillanates as β-lactamase inhibitors[2,6] and perhaps lead to applications with other enzymes.[7] Thus we present the kinetics of, and other observations pertinent to, the rearrangement of the 6-halopenicilloates (**2**) (this trivial name is used throughout for the 2-(4′-carboxy-5′,5′-dimethyl-2′-thiazolidinyl)-2-haloacetates) and certain of their derivatives, **4** and **9**. This is followed by a discussion of how the possible mechanisms fit these facts. We conclude that the most likely mechanism involves direct intramolecular nucleophilic attack by the thiazolidine sulfur atom on the alkyl halide, yielding a bicyclic episulfonium ion intermediate, which then collapses to product.

Scheme I

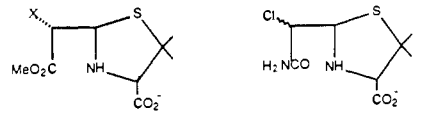




1aα (X = Cl) 1aβ (X = Cl)
1bα (X = Br) 1bβ (X = Br)
1cα (X = I) 1cβ (X = I)



2aα (X = Cl) 2aβ (X = Cl)
2bα (X = Br) 2bβ (X = Br)
2cα (X = I) 2cβ (X = I)



4aα (X = Cl) 9aα (6-α-Cl)
4bα (X = Br) 9aβ (6-β-Cl)

Experimental Section

Materials. 6α-Chloropenicillanic acid (**1a**α) and 6α-bromopenicillanic acid (**1b**α) were prepared by the method of McMillan and Stoodley.[8]

(1) McMillan, I.; Stoodley, R. J. *Tetrahedron Lett.* **1966**, 1205–1210.
(2) Pratt, R. F.; Loosemore, M. J. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 4145–4149.
(3) Knott-Hunziker, V.; Orlek, B. S.; Sammes, P. G.; Waley, S. G. *Biochem. J.* **1979**, 365–367.
(4) Knott-Hunziker, V.; Waley, S. G.; Orlek, B. S.; Sammes, P. G. *FEBS Lett.* **1979**, *99*, 59–61. Orlek, B. S.; Sammes, P. G.; Knott-Hunziker, V.; Waley, S. G. *J. Chem. Soc., Chem. Commun.* **1979**, 962–963.
(5) Cohen, S. A.; Pratt, R. F. *Biochemistry* **1980**, *19*, 3996–4003.
(6) von Daehne, W. *J. Antibiot.* **1980**, *33*, 451–452.
(7) Doherty, J. B.; Ashe, B. M.; Argenbright, L. W.; Barker, P. L.; Bonney, R. J.; Chandler, G. O.; Dahlgren, M. E.; Dorn, C. P., Jr.; Finke, P. E.; Firestone, R. A.; Fletcher, D.; Hagmann, W. K.; Mumford, R.; O'Grady, L.; Maycock, A. L.; Pisano, J. M.; Shah, S. K.; Thompson, K. R.; Zimmerman, M. *Nature (London)* **1986**, *322*, 192–194.

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Table I. ^1H NMR Spectral Data of 6-Halopenicillanates, 6-Halopenicilloates, and Derivatives

compound	solvent (^2H_2O)	Me_2	HC-3	HC-5	HC-6	CO_2Me	HCH=
1aα	HCO_3^-	1.49, 1.57	4.38	5.40 (d, J = 1.5 Hz)	5.07 (d, J = 1.5 Hz)		
1aβ	HCO_3^-	1.51, 1.62	4.33	5.73 (d, J = 4 Hz)	5.48 (d, J = 4 Hz)		
2aα	O^2H^-	1.26, 1.59	3.73	5.23 (d, J = 7 Hz)	4.43 (d, J = 7 Hz)		
4aα·HCl	HCO_3^-	1.24, 1.58	3.65	5.23 (d, J = 8 Hz)	4.64 (d, J = 8 Hz)	3.82	
4aα·HCl		1.41, 1.66	4.27	5.48 (d, J = 6 Hz)	5.02 (d, J = 6 Hz)	3.85	
4bα		1.39, 1.65	4.18	5.41 (d, J = 8 Hz)	(4.8–5.0)	3.86	
3	O^2H^-	1.21, 1.44	3.73				7.59
5	HCO_3^-	1.24, 1.51	3.83			3.72	7.85
6a	O^2H^-	1.27, 1.61	3.51	5.14 (d, J = 4 Hz)	4.83 (d, J = 4 Hz)		
6b	O^2H^-	1.27, 1.60	3.48	4.90	*a*		
9aα	N^2H_3	1.23, 1.58	3.61	5.12 (d, J = 9 Hz)	4.47 (d, J = 9 Hz)		
10aβ	N^2H_3	1.26, 1.61[b]	3.47	5.08	*a*		
10aα	N^2H_3	1.26, 1.59[b]	3.47	4.92	*a*		
11	N^2H_3	1.20, 1.48	3.79				7.61

[a] This species was only observed under circumstances where the C-6 hydrogen atom had exchanged with solvent deuterium. [b] The alternative assignment of these methyl groups is equally likely.

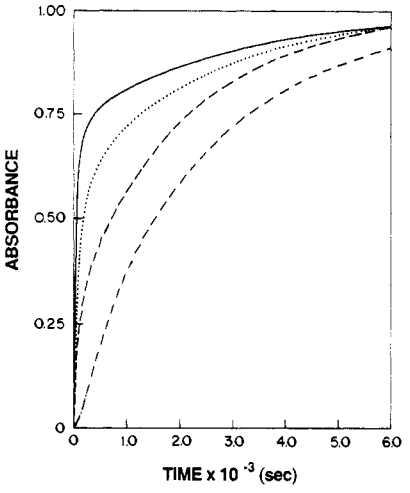

Figure 1. Measurements of absorption at 306 nm as a function of time for hydrolysis and rearrangement of **1aα** (0.12 mM solutions) in 0.01 M (-·-) and 0.05 M (- -) potassium hydroxide and of **1aβ** in 0.01 M (··) and 0.05 M (—) potassium hydroxide.

The sodium salts of the α- and β-isomers of 6-chloro-, 6-bromo-, and 6-iodopenicillanic acid were generously provided by Leo Pharmaceutical Products.

Methyl 6α-Chloropenicilloate (4aα) Hydrochloride. Redistilled acetyl chloride (2.7 mL) was added to 150 mL of methanol at room temperature, with stirring. The stirred solution was cooled to 0 °C, 2.0 g of 6α-chloropenicillanic acid was added, and the cooled mixture was stirred for a further 1.5 h. The volume was then reduced to 5 mL by rotary evaporation. To the resulting concentrate was added 150 mL of diethyl ether, and the resulting solution stood overnight at 0 °C. The colorless solid was removed by filtration and washed with diethyl ether. The solid product was redissolved in the minimum volume of cold methanol, and the ether precipitation was repeated. The final yield of dried material was 0.65 g (25%), mp 147–148 °C dec (lit.[9] mp 149 °C). The ^1H NMR spectrum of the product is given in Table I. Anal. Calcd for $C_9H_{15}Cl_2NO_4S$: C, 35.55; H, 4.95; Cl, 23.35; N, 4.60. Found: C, 35.90; H, 4.96; Cl, 22.82; N, 4.43. Methyl 6β-chloropenicilloate hydrochloride could not be prepared in this way, presumably because of its high rate of rearrangement.

Methyl 6α-bromopenicilloate (4bα) hydrochloride was prepared from 6α-bromopenicillanic acid by the above method with the difference that only 1 equiv of acetyl bromide was used. The product, mp 165–167 °C, was obtained in poor yield (ca. 5%); its ^1H NMR spectrum is reported in Table I.

Methyl (5*R*,6*R*)-Benzylpenicilloate. This compound, a colorless powder, was prepared by the method of Davis et al.[10]

Buffer materials and inorganic reagents were of commercial analytical quality. Deuteriated solvents and reagents were purchased from Aldrich Chemical Co.

Analytical Methods. Spectrophotometric measurements were performed with Gilford 2400 or Perkin-Elmer Lambda 4B spectrophotometers. Rates of reactions that were faster than could be determined by manual mixing methods were studied by means of a Durrum D-110 stopped-flow spectrophotometer; in particular the rates of rearrangement of 6β-chloropenicilloate were measured in this way. NMR spectra were obtained from either a Varian XL-200 or XL-400 spectrometer. The concentrations of 6-halopenicillanates and 6-halopenicilloates used in spectrophotometric measurements, including the kinetic studies, were routinely of the order of 10^{-4} M. All buffers were of ionic strength 1.0 (KCl) unless otherwise noted. Stock solutions of the reactants were prepared in water immediately prior to use. The formation of 3,6-dicarboxy-2,2-dimethyl-2,3-dihydro-1,4-thiazine from the 6-halopenicilloates was followed spectrophotometrically at 306 nm.[5] NMR studies routinely employed 10 mM concentrations of reactants. Sodium 3-(trimethylsilyl)-1-propanesulfonate was used as an internal standard.

Results

Reactions of 6-Halopenicillanic Acids in Alkaline Solution. ^1H NMR studies showed that in aqueous solution of pH 7.5–13.8 all 6-halopenicillanic acids were converted quantitatively to the dihydrothiazine **3**. (The ^1H NMR spectrum of **3** is reported in

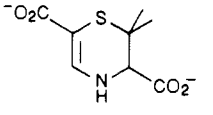

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Table I and is identical with that previously observed in this laboratory.[5]) Spectrophotometric observation of this reaction in hydroxide ion solution (0.01–1.0 M) produced data such as that shown in Figure 1 for **1aα** and **1aβ**. Clearly the reaction has more than one phase, and thus there must be an intermediate or intermediates. The rates of the earlier phase(s) of the reaction were apparently hydroxide ion dependent, while a final slow phase in each case was not. This slow phase could be conveniently isolated by observations in 0.5–1.0 M hydroxide ion where the initial phases were instantaneous (the "burst") under manual mixing conditions. Although the amplitude of the final slow phase was different in the two cases of **1aα** and **1aβ**, the reactions were both first-order and had the same rate constant [(4.5 ± 0.5) × 10^{-4} s^{-1}]; presumably, despite the differences seen in Figure 1, at least one common intermediate is generated by **1aα** and **1aβ**. Final spectra of the reaction mixtures of **1aα** and **1aβ** were identical, showing the presence of a species of λ_{max} 306 nm, ε 8200 M^{-1} cm^{-1}, which is the known[5] spectrum of **3**.

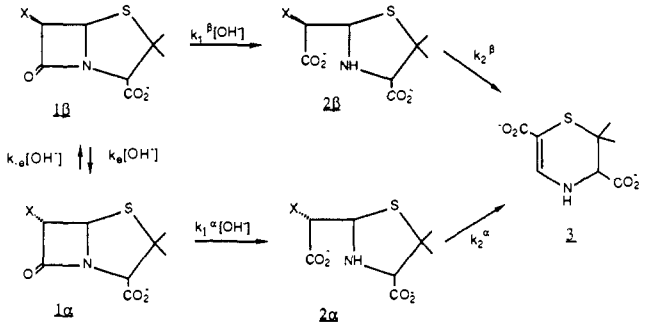
More extensive ^1H NMR observations over the time course of the reaction of **1aα** in dilute hydroxide solutions (0.01 M) demonstrated the presence, at short reaction times, of **1aα**, **1aβ**, **2aα**, and **3** (all spectra reported in Table I). At longer reaction times only **2aα** and **3** were present, and comparison of the rates observed

(8) McMillan, I.; Stoodley, R. J. *J. Chem. Soc. C* **1968**, 2533–2537.
(9) Stoodley, R. J. *J. Chem. Soc. C* **1968**, 2891–2894.
(10) Davis, S. B.; Willson, S. D.; Van Order, R. B. *J. Org. Chem.* **1948**, *13*, 682–689.

Table II. Rate constants for the Hydrolysis and Rearrangement of 6-Halopenicillanates

	k_e, s^{-1} M^{-1}	k_{-e}, s^{-1} M^{-1}	k_1^α, s^{-1} M^{-1}	k_1^β, s^{-1} M^{-1}	k_2^α, s^{-1}	k_2^β, s^{-1}
1a	0.35	0.11	0.15	0.53	$(4.5 \pm 0.5) \times 10^{-4}$	3.0 ± 0.5
1b					0.031	
1c					0.019	

Scheme II



by NMR with those from the spectrophotometric observations above made it clear that the slowly rearranging species was **2a**α. The identity of **2a**α follows from its generation as the only product (as observed by ^1H NMR in $^2H_2O/HCO_3^-$) of *B. cereus* β-lactamase II catalyzed hydrolysis of **1a**α. The appearance of **1a**β in the alkaline hydrolysis mixture is not surprising since it is known that 6-halopenicillins epimerize at C-6 in aqueous alkaline solution.[2,3,6,11] As a consequence of this epimerization reaction, **1a**β and **2a**α generated from **1a**α in ^2H_2O showed no C-6 hydrogen resonance (through exchange with solvent) and a singlet absorption for the hydrogen at C-5; the residual **1a**α had also undergone exchange at C-6. It should be noted, however, that **2a**α, generated enzymatically, did not exchange at C-6 in these hydroxide (O^2H^-) solutions; **2a**α does not epimerize at C-6 under these conditions. No resonance corresponding to **2a**β was ever observed, suggesting that this species, if formed, must rearrange to **3** very rapidly. That the latter is true was demonstrated by the addition of the *B. cereus* β-lactamase II to a solution of **1a**β in $^2H_2O/HCO_3^-$; an immediate ^1H NMR spectrum (3 min) showed the presence of **3** only. Reaction of **1a**β in dilute hydroxide solutions gave rise to reaction mixtures whose NMR spectra demonstrated the existence of **1a**α, **1a**β, **2a**α, and **3**; in ^2H_2O solutions the **1a**α and **2a**α generated had undergone hydrogen exchange at C-6.

At higher hydroxide ion concentrations (>0.05 M), ^1H NMR spectra taken 5 min after addition of either **1a**α or **1a**β to the base, showed the presence of significant amounts of **3**, apparently generated in a rapid burst, and **2a**α, which then slowly cyclized to **3**. The relative amounts of **3**, generated in the burst, and **2a**α differed, depending on whether **1a**α or **1a**β was the starting material (see below).

On the basis of these observations and those made in this laboratory and elsewhere[2-4,11,12] previously, Scheme II (X = Cl) seemed likely to serve as a useful framework for discussion of these results. In terms of Scheme II, the smallest rate constant is k_2^α. Since a lag in **3** formation is observed on starting with **1a**α (Figure 1), $k_1^\alpha[OH^-]$ and $k_e[OH^-]$ must be relatively small although larger than k_2^α; no lag is seen on starting with **1a**β since $k_2^\beta \gg k_1^\beta[OH^-]$ (this must be true since **2a**β never accumulates). The "burst" of **3**, observed in both the ^1H NMR and absorption experiments must correspond to **3** formed via **1a**β and **2a**β. In order to fit the spectrophotometric data of Figure I and similar data at the other hydroxide ion concentrations, quantitatively to Scheme II, it was useful to be able to constrict the values of some of the rate constants by knowledge of the ratio of the amount of starting material rearranging through **2a**α to that through **2a**β. Since k_2^α is so small with respect to the other rate constants at hydroxide ion concentrations 0.1–1.0 M, this ratio is simply obtained from the relative amounts of **3** formed in the slow phase (via **2a**α) and in the burst (via **2a**β). This ratio, r, was obtained spectrophotometrically, and was found not to change with hydroxide ion concentration between 0.1 and 1.0 M. The r values were different, however, depending on whether the starting material was **1a**α or **1a**β; $r_{1a\alpha} = 2.1 \pm 0.1$ (more of the reaction proceeds via **2a**α than **2a**β when **1a**α is the starting material) and $r_{1a\beta} = 0.37 \pm 0.02$ (more of the reaction proceeds via **2a**β when **1a**β is the starting material). A combination of the observation that r does not change with the hydroxide ion concentration with the fact that the burst becomes faster with increasing hydroxide (and the lag in **3** formation from **1a**α shorter) shows that the interconversion of **1a**α and **1a**β and their respective conversions to **2a**α and **2a**β must be all of the same order, greater than zero, in hydroxide ion concentration. All chemical precedent would suggest first order, as shown in Scheme II. The rate of rearrangement of **2a**α (formed either by addition of **1a**α to hydroxide ion solutions or enzymatically at lower pH) to **3** was found to be pH-independent between 7.5 (phosphate buffer) and 13.8 (1.0 M potassium hydroxide), as also shown in Scheme II (the same is assumed to be true of k_2^β). The rearrangement reaction was also not catalyzed by phosphate and carbonate buffer species (0.02–0.10 M) at pH 7.5 and 9.5, respectively.

Table III. Rate Constants for the Rearrangement of Derivatives of the 6-Halopenicilloates

derivative	conditions	$k_{obsd} \times 10^4$, s^{-1}
4aα	pH 7–8	1.10
4bα	pH 7–8	11.5
6a	0.1 M KOH	0.5
6b	0.1 M KOH	11.6
9aα	pH 7–9.5	0.82

Given the spectrophotometric data from 0.1–1.0 M hydroxide ion solutions for reactions of both **1a**α and **1a**β, the equations for the appearance of **3** with time (Appendix) according to Scheme II, values for the product formation ratios $r_{1a\alpha}$ and $r_{1a\beta}$ given above, equations for these r values (Appendix), and the result $k_2^\beta \gg k_1[OH^-]$ at all of the above $[OH^-]$, values for the other rate constants were determined by using a nonlinear least-squares routine.[13] These values are reported in Table II. These appear to agree with expectations: $k_1^\beta > k_1^\alpha$, evident from the NMR evidence described above and chemically reasonable since nucleophilic attack at the less hindered α-face of **1a**β should be more rapid than at either face of **1a**α, and $k_e > k_{-e}$, i.e. $K_e = k_e/k_{-e} > 1$, i.e. the α-isomer with less steric interaction between the 6-substituent and the 2β-methyl group is thermodynamically more stable.

The value of k_2^β could be determined through rapid enzymatic generation of **2a**β. Thus the RTEM-2 β-lactamase (2–10 μM) catalyzed the hydrolysis of **1a**β (100 μM) sufficiently fast (in the stopped-flow spectrophotometer) to enable determination of the rate of an enzyme-concentration-independent formation of **3**, presumably from **2a**β. These experiments yielded the value of k_2^β reported in Table II.

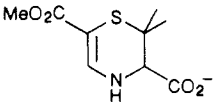
The other halides **1b**α, **1b**β, **1c**α, and **1c**β yielded similar reaction curves to those plotted for the chloride in Figure I, except the reactions were much faster. The final relatively slow first-order and hydroxide ion concentration independent steps were assumed, on the basis of the above results, to arise from the rearrangements of **2b**α and **2c**α and gave the rate constants reported as k_2^α in Table

(11) Loosemore, M. J.; Pratt, R. F. *J. Org. Chem.* **1978**, *43*, 3611–3613.
(12) Gensmantel, N. P.; McLellan, D.; Morris, J. J.; Page, M. I.; Proctor, P.; Randahawa, G. S. In *Recent Advances in the Chemistry of β-Lactam Antibiotics*; Gregory, G. I., Ed.; Royal Society of Chemistry: London, 1980; pp 227–239.

(13) Johnson, M. L.; Halvorson, H. R.; Ackers, G. K. *Biochemistry* **1976**, *15*, 5363–5371.

II. The total progress curves were not analyzed in detail to provide the other parameters. NMR observations on reaction mixtures at pH 9.0 showed the presence of both epimers of the reactants in solution, irrespective of which epimer was solely present initially. No hydrolyzed species **2b** or **2c** were observed, confirming the higher rearrangement rates of these species.

Reactions of the Methyl 6α-Halopenicilloates 4aα and 4bα in Basic Solution. In solutions with pH values between 7 and 8, **4aα** and **4bα** were observed to rearrange in a first-order, pH-independent fashion to **5** as the only product. The latter compound was identified by its ^{1}H NMR spectrum (Table I) and its absorption spectrum (λ_{max} 314 nm).[5] First-order rate constants of the rearrangement of **4aα** and **4bα** under these conditions are given in Table III.



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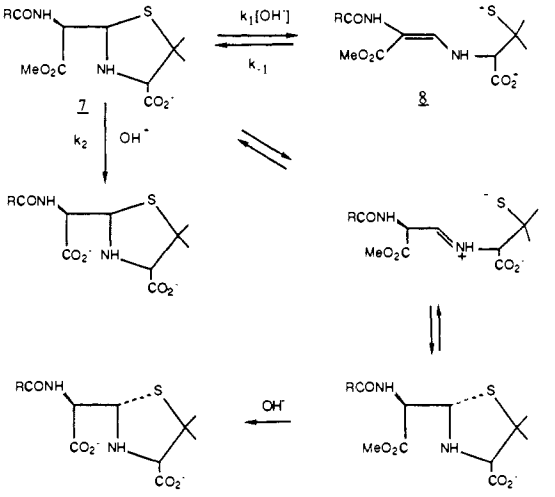
At higher pH, the reactions of **4aα** were more complicated. A ^{1}H NMR study of the reaction at pH 9.0 (0.1 M carbonate buffer) showed competing hydrolysis of the methyl ester yielding **2aα**, which then rearranged to **3**. Another compound, **6a**, was also observed in the spectra (Table I) and which also led to **3**. The same unknown compound was also seen in more highly alkaline solution. Thus, addition of **4aα** to deuteroxide ion solutions (0.1–1.0 M) yielded an immediate (ca. 3 min) spectrum of **2aα** and **6a**, in an approximately 3:1 ratio; again both yielded **3** on further reaction. One equivalent of methanol (δ 3.33) was also produced immediately, presumably from hydrolysis of the methyl ester. It was noticed from the spectrum of **2aα** in this hydrolysate that the C-6 hydrogen was not exchanged with solvent (^2H_2O), i.e. the two doublets of H-6 and H-5 (Table I) were present. In contrast, the spectrum of the other product, **6a**, had only a singlet absorption (δ 5.14) at chemical shifts higher than δ 4.0, suggesting exchange with solvent might have occurred on formation of **6a**. This conjecture was verified on carrying out the reaction in H_2O. A ^{1}H NMR spectrum of this product showed two doublets (J = 4 Hz) at δ 5.14 and 4.83. The significance of these observations, in concert with those on the reactions of methyl (5*R*,6*R*)-benzylpenicilloate (below) will be discussed below. The NMR observations also showed that the formation of **3** from **6a** was much slower, some 10 times, than from **2aα**. Similar observations could be made spectrophotometrically at 306 nm on addition of **4aα** to potassium hydroxide solutions, viz. no instantaneous burst of **3**, formation of **3** at the rate typical of **2aα**, and then a much slower reaction yielding **3**.

Similar spectrophotometric observations of a two-phased formation of **3** in hydroxide ion solutions were made with **4bα**. Here the amplitudes of the fast phase (corresponding to rearrangement of **2bα**, since the same rate constant was obtained as that when **1bα** was added to base) was about 5 times that of the slower phase (corresponding, presumably, to the rearrangement of **6b**). The rate constant associated with the slower phase was 1.16×10^{-3} s^{-1}. Addition of **4bα** to 0.1 M sodium deuteroxide allowed the observation of the NMR spectrum of **6b** (Table I).

Observations on the Reactions of Methyl (5*R*,6*R*)-Benzylpenicilloate in Alkaline Solution. Davis and Page[14] have described the transient formation of an intermediate absorbing around 280 nm on addition of methyl (5*R*,6*R*)-benzylpenicilloate, **7** (R = $PhCH_2$), to sodium hydroxide solutions (0.01–0.08 M). They interpret this observation in terms of Scheme III, where the absorbing species is suggested to be the enamine **8**. The products of the reaction are stated to be a mixture of (5*R*,6*R*)- and (5*S*,6*R*)-penicilloic acids. We have made similar observations of an intermediate under comparable conditions (0.02–0.10 M sodium hydroxide, μ = 1.0 (NaCl)) and have quantitatively fitted[13] Scheme III (steps 1 and 2) to the absorption changes (which are

Scheme III



similar to those reported by Davis and Page[14]); our results give the rate constants k_1 = 5.6 s^{-1} M^{-1}, k_{-1} = 4.3 s^{-1}, and k_2 = 34 s^{-1} M^{-1}. It was assumed in fitting these curves that the extinction coefficient of the transient enamine was 19 600 M^{-1} cm^{-1}.[15] Our results appear to differ from those of Davis and Page[14] in that ^{1}H NMR spectra of alkaline hydrolysis mixtures (0.1 M NaO^2H) show (5*R*,6*R*)-benzylpenicilloate to be the only immediate product (≥95%). The product spectrum is identical with that reported by Ghebre-Sellassie et al.[16] and to that obtained immediately on addition of β-lactamase to benzylpenicillin at neutral pH. The significance of this will be made clear below.

Similarly, addition of **4aα** to alkaline solutions also yielded transient species absorbing maximally around 280 nm, whose formation and decay occurred prior to any significant formation of **3**. Both formation and decay (presumably through methyl ester hydrolysis of **4aα**) of this species were more rapid than those of **8**, as would be expected on the basis of the electronic effects of the C-6 substituents. Enamine species are thus probably present under the circumstances of formation of **6a** from **4aα**. Comparable observations have been made in solutions of **4aα** methyl ester in sodium methoxide/methanol by McMillan and Stoodley.[1] Thus it seems reasonable that the formation of **6a** involves the intermediacy of the enamine analogous to **8**.

We propose that these observations can be explained as follows. The products of reaction of **4aα** in alkaline solution are **2aα** and another chloropenicilloate diastereoisomer, **6a**. It is clear that **6a** must have *S* stereochemistry at C-5, since epimerization of **2aα** at C-6 alone would yield **2aβ**, which rearranges very rapidly and thus cannot be **6a**. The problem is that of the stereochemistry of **6a** at C-6. On addition of **4aα** to base, methyl ester hydrolysis competes with enamine formation. We propose that under alkaline conditions, the time available prior to ester hydrolysis permits only reversible elimination across the C_5–C_6 bond to yield the enamine, but not protonation of the enamine at C-6 (by water presumably) to epimerize C-6 independently of the elimination reaction. The elimination reaction should require a trans relationship between the hydrogen on C-6 and the leaving group sulfur. Thus, methyl (5*R*,6*R*)-benzylpenicilloate should yield only itself or methyl (5*S*,6*S*)-benzylpenicilloate (cf. Scheme III). That none of the latter is seen is reasonable since it is one of the least stable of the benzylpenicilloate diastereoisomers (making up something less than 5% of the total at equilibrium[17]). The fact that none of the most stable isomer (5*S*,6*R*) was observed shows that kinetic factors must also be important in dictating the product composition, as proposed above. On this basis it is suggested that the compound **6a**, obtained as the only product derived from recyclization of the

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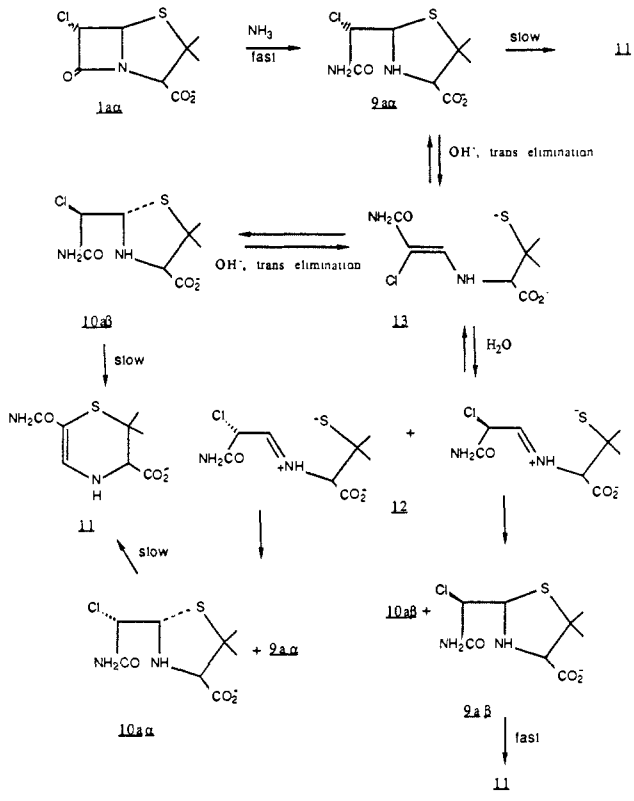
(15) Schwartz, M. A.; Delduce, A. J. *J. Pharm. Sci.* **1969**, *58*, 1137–1139.

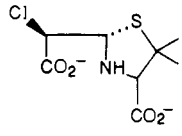
(16) Ghebre-Sellassie, I.; Hem, S. L.; Knevel, A. M. *J. Pharm. Sci.* **1984**, *73*, 125–128.

(17) Busson, R.; Claes, P. J.; Vanderhaeghe, H. *J. Org. Chem.* **1976**, *41*, 2556–2561.

Scheme IV



enamine to the thiazolidine on addition of **4aα** to base (the only product since the **2aα** generated did not have its C-6 proton exchanged with solvent and hence presumably could not have derived from the enamine), should be the 5*S*,6*R* isomer.



6a

Reactions of the Amides of 2aα and 2aβ. ^{1}H NMR observation at 25 °C of a solution of **1aα** (10 mM) in concentrated ammonia solution (5.6 M N^2H_3 in ^2H_2O) showed that it was converted instantaneously (complete in <1 min) into a single product whose spectrum (Table I) was interpreted as that of **9aα** (Scheme IV), from direct β-lactam ammonolysis.[18] Over an hour the doublet at δ 4.47 disappeared and the doublet at δ 5.12 changed into a singlet. This can be interpreted in terms of exchange of the C-6 hydrogen with solvent. During the same time period two other compounds arose, whose spectra (Table I) are interpreted in terms of the epimers **10aβ** and **10aα** (Scheme IV). Also appearing was a fourth compound **11**, whose spectrum (Table I) strongly suggested the dihydrothiazine amide (Scheme IV). The compounds **9aα**, **10aα**, and **10aβ** then disappeared in an apparently concerted fashion, yielding **11**; the concerted loss over several hours of all three suggested they were present in a close to equilibrium situation, in the approximate ratio 2:1:1. The final absorption spectrum of **11**, with absorption at 311 nm (ϵ = 9400 M^{-1} cm^{-1}) supports the structure given in Scheme IV.

These observations, in the light of the results described in prior sections, are interpreted in terms of Scheme IV, where the initially formed amide undergoes epimerization at both C-5 and C-6, the latter leading to solvent exchange. Epimerization at C-6 occurs here, independently of that at C-5 (via the imines **12**, presumably) and in contrast to the situation with the ester **4aα**, since the amide, unlike the ester, is stable to the hydrolysis competing with the formation of **12**. The assignment of the respective structures to **10aα** and **10aβ** is tentative, that of **10aβ** is based only on the closer resemblance of its ^{1}H NMR spectrum to that of **6a** (Table I). The assignment of **10aα** is then by default since **9aβ**, the remaining epimer, rearranged to **11** instantaneously. The latter is clear since addition of ammonia to a solution of **1aβ** yielded only **11** instantaneously.

These observations were also complemented by spectrophotometric measurements. The compound **9aα** was generated in concentrated ammonia as described above, the solution was rapidly (1–2 min) neutralized with hydrocholoric acid, and small aliquots were added to cuvettes containing phosphate (pH 7.5) and carbonate (pH 9.5) buffers. The slow formation of **11** was followed at 311 nm, giving the rate constants included in Table III. Since it was clear from the ^{1}H NMR results that the rearrangement was faster at higher pH, some observations were made in potassium hydroxide solutions (0.10–1.0 M) also. Here the rearrangement appeared to be hydroxide ion catalyzed. Furthermore, observation at 280 nm revealed transient formation of what is presumably the enamine **13**. This transient then decayed with the same apparent rate constant as the formation of **11**.

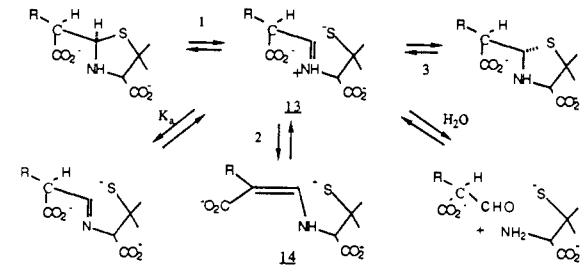
Solvent Effects on the Rate of Rearrangement of 2aα. The effect of lower solvent dielectric constant on the rate of rearrangement of **2aα** was measured in ethanol/water and dioxane/water mixtures in 0.1 M potassium hydroxide. The results are shown in Table IV.

Table IV Solvent Effects on the Rate of Rearrangement of **2aα** in 0.1 M KOH (μ = 1.0, KCl)

solvent	ϵ	$k_{absd} \times 10^4$, s^{-1}
water	78.5[a]	4.5
40% (w/w) EtOH	55.2[a]	1.6
30% (w/w) dioxane	53.3[b]	2.4

[a] Reference 19. [b] Reference 20.

Scheme V



Salt Effects on the Rate of Rearrangement of 2aα. A positive salt effect was observed on the rate of rearrangement of **2aα** in solutions of potassium chloride and sodium perchlorate (both 0–1.0 M). Plots of the observed rate constant versus salt concentration were linear with slopes of 2.43 × 10^{-4} s^{-1} M^{-1} and 1.74 × 10^{-4} s^{-1} M^{-1}, respectively; the rate constant at zero ionic strength was 2.72 × 10^{-4} s^{-1}.

There was little indication of specific anion effects. For example, essentially no difference was observed in 0.1 M sodium hydroxide between 0.9 M sodium iodide and 0.9 M sodium perchlorate. Sodium thiosulfate (1 M) had little effect either on rates in 0.1 M phosphate buffer, pH 7.5, or in 1 M potassium hydroxide.

Hydrogen Isotope Effects. The kinetic solvent isotope effect (k_{H_2O}/k_{D_2O}) on the rearrangement of **2aα** (in 0.1 M potassium hydroxide) was determined to be 1.14 ± 0.02. 6-[^{2}H]-**2aα** was generated from addition of **1aβ** to a solution of 0.1 M potassium deuteroxide in deuterium oxide. The kinetic isotope effect of this substitution on the rearrangement of **2aα**, measured in 0.1 M potassium hydroxide solution, was 1.03 ± 0.02.

Ring Opening of Thiazolidines. The first step in many reactions of thiazolidines, including hydrolysis, is the opening of the ring, with formation of a thiolate-immonium zwitterion **13** (step 1,

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(20) Critchfield, F. E.; Gibson, J. A., Jr.; Hall, J. L. *J. Am. Chem. Soc.* **1953**, *75*, 1991–1992.

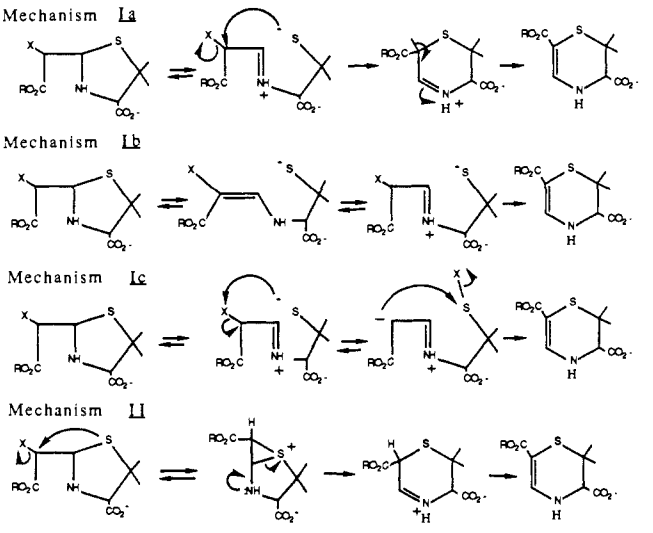
Scheme V).[21-23] The position of this equilibrium and the rates of its attainment vary widely with substituents on the ring,[22,23] and thus generalizations cannot be readily made as to whether a given reaction proceeds by reaction of the open or closed form; thiazolidines formed from penicillamine however appear to be very stable.[23,14] In general, the imines do not accumulate in solution with respect to the closed thiazolidine or its hydrolysis products, except in cases where a conjugating substituent is present at C-2 (thiazolidine numbering).[22,23] Evidence for equilibration of the imine with the enamine **14** (step 2, Scheme V) under some circumstances is demonstrated by exchange of α protons of C-2 substituents with those of solvent.[22]

The rate of ring opening of penicilloates is generally believed to be given by the (measurable) rate of epimerization at C-5 (step 3, Scheme V);[14,16,24] that of (5*R*,6*R*)-benzylpenicilloate is 2.0×10^{-5} s^{-1} at 25 °C.[16] Except perhaps in strongly alkaline solution,[14] there is no evidence for the enamine since the protons at C-6 do not exchange with ^2H_2O over the time scale of the epimerization; thus apparently $k_{-1} + k_3 \gg k_2$. Direct elimination of $^-$SR across the C_5–C_6 bond to form **14** also cannot be facile in less basic solutions than 1.0 M hydroxide ion[14] (cf. penicilloate esters and amides and the discussion above). Similar results have been obtained with (5*R*,6*R*)-aminopenicilloate (Scheme V, R = NH_2),[25] although here the epimerization at C-5 and hence the thiazolidine ring opening is considerably faster, presumably because of the lesser electron withdrawing power of the C-6 substituent. On this basis one would expect that the ring opening of 6-halopenicilloates to be considerably slower than that of the benzylpenicilloate. Certainly no sign of epimerization at C-5 or proton exchange at C-6 was observed prior to dihydrothiazine formation in even **2a**α, the most slowly rearranging halide. Thus, if in fact these epimerization rates are indeed the rates of thiazolidine ring opening, the rearrangements of **2**β, **2b**α, and **2c**α, all of which are much faster than that of **2a**α, cannot go through a thiazolidine-ring-opened species, i.e. for them, mechanisms I (see Discussion) cannot be correct.

There is, however, the possibility that thiazolidine ring opening in penicilloates is actually faster than epimerization at C-5. This could occur and be undetected if the single bond rotation and solvent reorganization necessary for epimerization were slower than ring closure. This possibility could in principle be detected by other ways of trapping the open form (such as by the use of an intramolecular alkyl halide as a thiolate trap(!)). Such speculation is fueled by long-established indications of rapid thiol trapping in aqueous thiazolidine solutions. Many early experiments[26] showed reactions of thiazolidines with a variety of thiol-specific reagents and were usually interpreted in terms of rapid thiazolidine–imine thiol equilibria. In certain cases[27] this interpretation is probably correct, but is it for penicilloates?

Of the reactions referred to above, probably the best known is the reaction of thiazolidines with iodine. Iodine reacts rapidly, essentially instantaneously, with penicilloates, which is the basis of the classical starch–iodine assay for them. The route to the products[28] clearly involves, at some stage, the opening of the thiazolidine ring and oxidation of sulfur. Our observations on initial rates of this reaction with benzylpenicilloate[29] show the

Scheme VI



reaction to be first order in both iodine and penicilloate, i.e. that rates were not limited by thiazolidine ring opening, and much faster, completed in seconds, than the epimerization rates. There is, however, no need to assume that the reaction necessarily proceeds through the intermediacy of the imine thiolate. The rapid oxidation of sulfides by iodine, particularly in the presence of buffer and intramolecular (carboxylate and amine) catalysts is well documented.[30] In the present case of thiazolidines it seems reasonable that iodine oxidation might proceed by preequilibrium formation of an iodosulfonium ion,[30] i.e. without prior ring opening; intramolecular carboxylate catalysis might well also occur in penicilloates.

Also well-established is the rapid reaction of mercuric ion with penicilloates to produce mercuric penamaldates.[31] Our observations of this reaction with benzylpenicilloate[32] confirm that it too is much faster than C-5 epimerization. We found it to be first order in mercuric chloride and zeroth order in benzylpenicilloate, suggesting that reaction occurred through preequilibrium formation of a mercuric complex. Again, initial ring opening is not needed to explain the reaction; the reagent reacts first with the thiazolidine sulfur atom, and subsequent reaction requires ring opening. These ideas also accommodate the observations that *N*-acylthiazolidines do not yield ring opened products with either iodine or mercuric ion, which have often been interpreted in terms of the inability of these compounds to spontaneously ring open. In contrast to these observations, the reaction of ferricyanide with benzylpenicilloate[33] proceeded with rates comparable to those of epimerization at C-5. Under the same conditions no reaction was observed with intact benzylpenicillin, and the reaction with penicillamine was instantaneous.

We conclude that although it seems difficult to completely prove that the thiazolidine ring opening of penicilloates is not fast with respect to epimerization at C-5, there is no evidence that it is. The

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(29) The reaction was followed spectrophotometrically by the decrease in triiodide absorption at 355 nm in solutions containing iodine (20–50 μM), benzylpenicilloate (1–2 mM), and iodide (0.01–0.05 M) in 0.05 and 0.1 M phosphate buffers (μ = 1.0 (NaCl)) at pH 7.0 at 25 °C.
(30) Higuchi, T.; Guensch, K.-H. *J. Am. Chem. Soc.* **1966**, *88*, 3874–3875. Higuchi, T.; Guensch, K.-H. *J. Am. Chem. Soc.* **1966**, *88*, 5486–5491. Doi, J. T.; Musker, W. K.; deLeeuw, D. L.; Hirschon, A. S. *J. Org. Chem.* **1981**, *46*, 1239–1243. Doi, J. T.; Musker, W. K. *J. Am. Chem. Soc.* **1981**, *103*, 1159–1163. Hirschon, A. S.; Doi, J. T.; Musker, W. K. *J. Am. Chem. Soc.* **1982**, *104*, 728–730.
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(32) The reaction was followed spectrophotometrically by the increase in absorption at 276 nm, due to the mercuric penamaldate, in solutions containing mercuric chloride (2.5–5.0 μM) and benzylpenicilloate (25–100 μM) in 0.1 M carbonate buffer at pH 9.0 at 25 °C.
(33) The reaction was followed spectrophotometrically at 420 nm (ferricyanide absorption) in solutions containing potassium ferricyanide (4–8 mM) and benzylpenicilloate (40–80 mM) in 0.1 M phosphate buffer (μ = 1.0, NaCl) at pH 7.0 at 25 °C.

Table V. Evaluation of the Mechanisms

criterion	mechanism[a-c]						
	I_{a1}	I_{a2}	I_{a3}	I_{b1}	I_{b2}	I_{c2}	II
1. pH profile	+	+	+	-	-	+	+
2. leaving group effect	-	-	+	-	?	+	+
3. stereochemistry	-	-	-	-	-	-	+
4. α-carboxyl derivative	+	-	-	-	?	-	+
5. ring-opening rate	-	-	-	+	+	-	+
6. exchange/epimerization	+	+	-	+	-	+	+
7. isotope effects	+	-	+	-	-	+	+

[a] Refer to Scheme VI. [b] I_{a1}: mechanism I_a with rate-determining thiazolidine ring-opening. I_{a2}: mechanism I_a with rate-determining imine isomerization via enamine. I_{a3}: mechanism I_a with rate-determining cyclization of open-chain imine. I_{b1}: mechanism I_b with rate-determining enamine formation. I_{b2}: mechanism I_b with rate-determining imine formation. I_{b3}: mechanism I_b with rate-determining cyclization of open-chain imine. Omitted from table since implications are as for I_{a3}. I_{c1}: mechanism I_c with rate-determining thiazolidine ring-opening. Omitted from table since implications are as for I_{a1}. I_{c2}: mechanism I_c with rate-determining nucleophilic displacement of halide. [c] (+) The observation is consistent with the mechanism. (-) The observation is not consistent with the mechanism. (?) The decision is not clear-cut.

most unambiguous evidence (from the ferricyanide experiments) suggests that it is not. If it is not, then the conclusion mentioned above stands, viz. mechanisms I (see Discussion) cannot be correct for many of the 6-halopenicilloates.

Discussion

The detailed analysis of the alkaline hydrolysis of **1aα** and **1aβ** presented in the Results shows that Scheme II obtains. Semiquantitative observations suggest that this scheme is also applicable to the bromo and iodo analogues **1b** and **1c**. Thus, as described in the Results, it was possible to obtain rate constants for the rearrangement of the 6-halopenicilloates **2aα**, **2bα**, and **2cα** to the dihydrothiazine **3** (Table II). β-Lactamase-catalyzed generation of **2aβ** allowed its rate of rearrangement to be determined also (Table II). The latter method could not be used to generate **2bβ** and **2cβ**, indicating, as would be anticipated on the basis of the results with the 6α-halopenicilloates, that **2bβ** and **2cβ** rearranged considerably more rapidly than **2aβ** (some 50 times perhaps, on the basis of the 6α-halopenicilloate data, yielding rate constants of around 150 s^{-1}). Synthesis of the methyl esters of **1aα** (**4aα**) and **1bα** (**4bα**) and of the amide of **1aα** (**9aα**) allowed determination of the rates of rearrangement of these derivatives also to the corresponding dihydrothiazine derivatives **5** and **11** (Table III).

The mechanisms previously proposed for the 6-halopenicilloate rearrangement (Scheme VI) can be usefully subdivided into two groups, one involving initial ring opening of the thiazolidine (mechanism I), and the other not (mechanism II). Of these, I_b and II were initially considered by McMillan and Stoodley,[1] who preferred I_b (see below). Subsequently, in relationship to the β-lactamase inhibition studies, mechanisms I_a,[5] I_b,[34] and I_c[35] were put forward, although with little or no evidence. Recent reviews of these reactions have tended to emphasize mechanisms I_a and I_b.[12,36]

The experimental observations from this work, described in the Results, allow the various mechanisms to be critically evaluated. The criteria for judgment arising from these experiments and which are sufficient to decide the case of the 6-halopenicilloates are as follows:

1. The rates of rearrangement reactions are pH-independent between pH 7.5 and pH 13.8.

2. The rates of rearrangement of the bromo and iodo compounds are significantly greater than those of the chloro compounds.

3. The rate of rearrangement of the (5*R*,6*R*)-chloropenicilloate (**2aβ**) was much greater than that of the 5*R*,6*S* (**2aα**) and 5*S*,6*R* (**6a**) isomers. On the basis of the results with the amides, the rate of rearrangement of the remaining isomer (5*S*,6*S*) is probably much slower than that of **2aβ** also.

4. The rates of rearrangement of the 6-halopenicilloates **2aα** and **2bα** were somewhat greater than those of the corresponding methyl esters (**4aα** and **4bα**) and amide (**9aα**).

5. The rate of spontaneous opening of the thiazolidine ring in penicilloates is in all likelihood smaller than the rearrangement rates of most of the 6-halopenicilloates.

6. Rearrangement of **2aα** (and **4aα**) occurs more rapidly than exchange of the hydrogen atom on C-6 with solvent; no exchange is observed. No epimerization at either C-5 or C-6 is observed either.

7. No primary hydrogen isotope effect on the rate of rearrangement of **2aα** is observed when deuterium is incorporated either at C-6 of **2aα** or in the solvent water.

The decisions reached for each mechanism with respect to each of these criteria on simple inspection of the mechanisms is given in Table V. *It was assumed that the mechanism of rearrangement of all of the 6-halopenicilloates* (although not necessarily their carboxyl derivatives; see below) *is the same*, i.e. one of the mechanisms I and II applies to all. For each mechanism, however, the possibilities of different rate-determining steps were considered.

In general, those mechanisms where the first step is not rate-determining have difficulties with the exchange criterion or the leaving group effect. The exception is I_{c2} whose main problem, apart from the question of the rate of thiazolidine ring opening, is that of the acyl derivatives—the ester and amide should provide better leaving groups from the halogen than does carboxylate. A variant of I_{c2} where thiazolidine ring opening does not occur and the reaction is proposed to proceed by direct attack of the thiazolidine sulfur on halogen,[35b] appears to have the same difficulty, even if the stereochemistry of such a displacement were possible. Mechanisms I_a and I_c have the additional problem, not sufficiently taken into account in Scheme VI, of geometrical isomerization around the imine double bond. The *E* isomer, which should be the predominant (with respect to the *Z* isomer) product on ring opening of the thiazolidine, must presumably isomerize to the much less stable *Z* form[37] before cyclization of the open imine is possible. Although there are several possible mechanisms for this isomerization, it is not clear that any one of them, either direct rotation or inversion of the imine,[38] reversible enamine formation,[39] or reversible hydroxide or water addition to C-5[40] (which would

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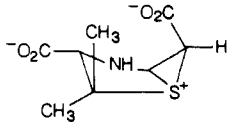
probably also lead to fragmentation[40]) would be rapid enough to accommodate the observed rates, particularly of the 6β-halopenicilloates. Mechanisms where the isomerization is rate-determining, e.g. I_{a2} (Table V) have difficulty with many of the above criteria.

Inspection of Table V suggests that each of the mechanisms has at least one very negative feature except mechanism II. Mechanism II accounts well for the leaving group effect since S_N2 type displacement reactions of alkyl bromides and iodides are invariably much more rapid than of chlorides; the relative reactivity of bromides and iodides varies however.[41] The meeting of the stereochemical criterion—just why the (5*R*,6*R*)-6-halopenicilloates rearrange so much more rapidly than the others—is perhaps not obvious but may well be the strongest argument in favor of mechanism II. The intermediate episulfonium ions derived from **2aα** (**15**) and **2aβ** (**16**) are shown, where the rings are seen from



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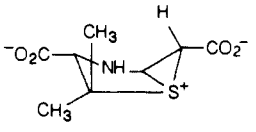
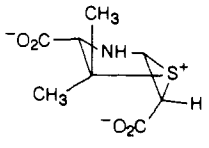


16

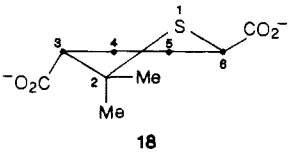
the side. It is assumed that the halide displacement is a normal S_N2 reaction with in-line geometry.[42] It seems likely that much greater unfavorable steric interactions between the endo substituent at C-6 and the β-methyl group will occur in **15** (and the associated transition states) than in **16**. This fact may well explain the difference in rates of rearrangement of **2aα** and **2aβ**. It appears that the (5*S*,6*R*)-6-chloropenicilloate (**6a**) rearranges even more slowly than **2aα**. This situation would be complementary to that of **2aα**, except that the unfavorable steric interaction in this case would be buttressed by the α-carboxylate group at the C-3 position of the thiazolidine ring, **17**, thus leading perhaps to the even lower



17

rates. The results of the amide experiments also suggest that the rearrangement of the remaining isomer (5*S*,6*S*) would be significantly slower than that of **2aβ**. It seems possible that this too could arise from the greater steric interactions on the α side of the ring.

In the initially formed 6-membered cyclic imine, these steric interactions, those of **15** for example, can be minimized in an appropriate conformation of the ring, e.g. **18**. Presumably an

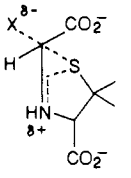


18

open-chain imine on cyclizing could avoid the unfavorable interaction of **15**–**17** enforced by the geometry of the fused rings, and thus, if the cyclization was rate-determining, although the leaving group effect might be seen in the rates, the steric effect would not (mechanisms I_{a3}, I_{b3}).

The rather unexpected[43] (by most mechanisms) greater reactivity of the carboxylates than their corresponding methyl esters or amides might also reflect these steric issues to some extent, although a perhaps more important contribution might come from electrostatic stabilization of the episulfonium ion intermediate and the transition state leading to it by the adjacent carboxylate anion.

A variant of mechanism II, where the collapse of the ion to products is rate-determining, can be also excluded because of the absence of an inhibitory common ion effect. Indeed the absence of halide exchange, even in 1 M iodide, and the total lack of effect of a very powerful nucleophile such as 1 M thiosulfate suggests that collapse of the sulfonium ion is very efficient. The collapse is presumably brought about by action of the lone pair on nitrogen and it may be that the reaction is to some extent concerted, i.e. with a transition state as in **19**. It seems possible to achieve a suitable thiazolidine conformation where the lone pair on nitrogen is close to antiperiplanar to the C_5–S bond (**20**).



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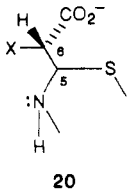

20

There may seem, at first, good reason to believe that α-carboxylic acid derivatives of 6-halopenicilloates can employ a mechanism other than II for the rearrangement under some circumstances. At lower pH (7–8) **4aα** (and **9aα**) and **4bα** rearrange at rates that are pH independent and similar to those of **2aα** and **2bα**, respectively. It also seems clear that **9aβ** rearranges much faster than **9aα**. Thus, under these conditions, mechanism II appears to obtain (at least to the extent that it does for the carboxylates). However at high pH the rate of rearrangement of the amide **9aα** is accelerated by hydroxide ion, absorption spectra indicate the transient presence of the enamine **13** (Scheme IV), and as shown by NMR spectra both exchange and epimerization at C-5 and C-6 occur in alkaline solution. These observations point to a mechanism such as I_{b2}. Indeed it was the observation of such base catalysis that led McMillan and Stoodley[1] to favor mechanism I_b. Despite these indications of a I_{b2} mechanism at high pH, it is still likely that the mechanism of the actual rearrangement of 6-halopenicilloate derivatives such as **7aα** is that of II. This would occur, if, as is likely,[14] cyclization of the open imine **12** to the reactive (5*R*,6*R*)-6-chloropenicilloate amide **9aβ** is faster than direct formation of the 6-membered ring. The catalytic effect of the base then would be not so much to produce rapidly cyclizing acyclic species but to catalyze formation of the rapidly rearranging (by mechanism II) (5*R*,6*R*) species. This catalysis was not seen in the esters **4aα** and **4aβ** since, although enamine formation was observed, a subsequent step, probably protonation of the enamine to form the imine, must have been slower than ester hydrolysis.

Thus the weight of the evidence, for the 6-halopenicilloates at least, is in favor of mechanism II. This mechanism would then also apply to their α-carboxylic acid derivatives at low pH (7–10) and probably also at high pH. Such a mechanism has firm precedent in related systems. β-Halo sulfides have long been known to solvolyze by way of episulfonium ions.[44] Goering and Howe[42] demonstrated the intermediacy of a [1,3]-fused episulfonium ion in the solvolysis of *cis*-2-chlorocyclohexyl phenyl sulfide. Amines are also well known to participate in the displacement of β-halides with the intermediacy of aziridines or aziridium ions. A pertinent example here would be the ring expansion of 1-ethyl-2-(chloromethyl)pyrrolidine to 1-ethyl-3-chloropiperidine,[45] a reaction that has much in common with the presently considered one and which demonstrates the important influence of the thiazolidine nitrogen atom in dictating the final outcome in the latter. A more recent example demonstrates this type of participation by a thiazolidine nitrogen in the rearrangement of a penicillanyl alcohol derivative.[46] It should be noted that although the currently considered reaction may in principle

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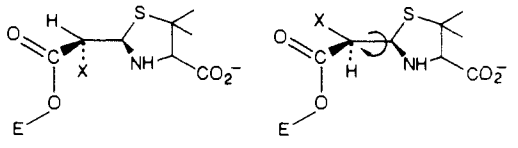
proceed through nitrogen rather than sulfur participation in a mechanism analogous to II, in fact it does not since this alternative should lead to a dihydrothiazine-3,5-dicarboxylate rather than the observed 3,6-derivative; intramolecular participation by a β-sulfide is generally more effective than by a β-amine.[47] The effect of perturbants on the current reaction, viz. the small positive salt effect,[42,44] the positive dielectric effect,[44,48] and the small secondary isotope effect at C-6,[49] are all in accord with, but do not in themselves prove, mechanism II. The absence of the well-documented common-ion effect (or external ion return) and the trapping of the episulfonium ions by other strong nucleophiles[44] can be ascribed, as noted above, to the efficient intramolecular and perhaps concerted participation of the thiazolidine nitrogen lone pair. The rate constant measured here for the rearrangement of **2aβ** appears to be considerably greater than observed previously for acyclic β-chloro sulfides under comparable conditions.[44,48] Contributing to this difference would probably be the entropic effect of the absence of C_5–S rotation in the penicilloates and perhaps the concerted assistance of the thiazolidine nitrogen lone pair.

These experimental results demonstrate why 6β-halopenicillanates are more effective than 6α-halopenicillanates as β-lactamase inhibitors[2,3] (another factor of course is that the former compounds also have the stereochemistry of natural penicillins, but this is in itself probably insufficient since 6α-penicillins do covalently interact with certain β-lactamases; one would thus expect the 6β-halopenicillanates to be more effective than the 6α-compounds with other enzymes also) and why the bromo and iodo derivatives are much more effective than the chloro.[6] Clearly the acyl enzymes generated from 6β-bromo- or 6β-iodopenicillanates will partition in favor of rearrangement and thus enzyme inactivation rather than hydrolysis more favorably than any others. If mechanism II does obtain in such acyl enzyme rearrangements, however, and there is at present no reason to believe that it does not, one proviso must be borne in mind before the current results can be taken as a general indication of inhibitory effectiveness. On formation of the acyl enzyme it is the 6α-halopenicilloyl derivatives that are spatially arranged appropriately for the in-line S_N2 displacement of mechanism II (**21**) (and despite the fact that the above discussed steric factors will cause the rearrangement to be slow) rather than the 6β-halo derivatives (**22**).



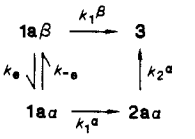
21 **22**

In order for the latter to rearrange, either the thiazolidine ring must rotate around the C_5–C_6 bond or the enzyme must move. The facility of these motions will strongly influence the effectiveness of 6β-halopenicillanates as inhibitors in an absolute sense and relative to the 6α-halopenicillanates. The greater effectiveness of 6β-bromo- and 6β-iodopenicillanate as inhibitors of class A β-lactamases than of class C β-lactamases[50] may reflect these issues, for example.

Acknowledgment. We are grateful to Drs. W. O. Godtfredsen and W. von Daehne of Leo Pharmaceutical Products for help in our obtaining the sodium halopenicillanates. Financial support for this work was provided by the National Institutes of Health.

Appendix

The kinetic scheme and equations used to fit the data from the reaction of **1aα** and **1aβ** in alkaline solution are as follows:



$$\frac{d[\mathbf{1a}\alpha]}{dt} = k_e[\mathbf{1a}\beta] - k_{-e}[\mathbf{1a}\alpha] - k_1^{\alpha}[\mathbf{1a}\alpha]$$

$$\frac{d[\mathbf{1a}\beta]}{dt} = k_{-e}[\mathbf{1a}\alpha] - k_e[\mathbf{1a}\beta] - k_1^{\beta}[\mathbf{1a}\beta]$$

$$\frac{d[\mathbf{2a}\alpha]}{dt} = k_1^{\alpha}[\mathbf{1a}\alpha] - k_2^{\alpha}[\mathbf{2a}\alpha]$$

$$\frac{d[\mathbf{3}]}{dt} = k_1^{\beta}[\mathbf{1a}\beta] + k_2^{\alpha}[\mathbf{2a}\alpha]$$

Simultaneous solution of these equations yields:

(i) With only **1aα** as starting material i.e. [**1aβ**] = 0 at zero time

$$[\mathbf{3}]/C_0 = A(e^{\lambda_1 t} - 1) + B(e^{\lambda_2 t} - 1) + C(e^{\lambda_3 t} - 1) + D(e^{\lambda_4 t} - 1) + E(1 - e^{-k_2^{\alpha} t})$$

where

$$A = k_2^{\alpha}k_1^{\alpha}(\lambda_2 + k_{-e} + k_1^{\alpha})/\lambda_1(\lambda_2 - \lambda_1)(k_2^{\alpha} + \lambda_1)$$

$$B = -k_2^{\alpha}k_1^{\alpha}(\lambda_1 + k_{-e} + k_1^{\alpha})/\lambda_2(\lambda_2 - \lambda_1)(k_2^{\alpha} + \lambda_2)$$

$$C = k_1^{\beta}k_{-e}/\lambda_3(\lambda_3 - \lambda_4)$$

$$D = -k_1^{\beta}k_{-e}/\lambda_4(\lambda_3 - \lambda_4)$$

$$E = -k_1^{\alpha}[(\lambda_2 + k_{-e} + k_1^{\alpha})/(k_2^{\alpha} + \lambda_1) - (\lambda_1 + k_{-e} + k_1^{\alpha})/(k_2^{\alpha} + \lambda_2)]/(\lambda_2 - \lambda_1)$$

$$\lambda_1, \lambda_2 = (-a \pm \sqrt{a^2 - 4b})/2$$

$$\lambda_3, \lambda_4 = (-a \pm \sqrt{a^2 - 4b})/2$$

$$a = k_1^{\alpha} + k_1^{\beta} + k_e + k_{-e}$$

$$b = k_1^{\alpha}k_e + k_1^{\alpha}k_1^{\beta} + k_1^{\beta}k_{-e}$$

[**3**] is the concentration of **3** at any time, arising from an initial concentration C_0 of **1aα**

(ii) With only **1aβ** as starting material, i.e. [**1aα**] = 0 at zero time

$$[\mathbf{3}]/C_0 = F(e^{\lambda_1 t} - 1) + G(e^{\lambda_2 t} - 1) + H(e^{\lambda_3 t} - 1) + I(e^{\lambda_4 t} - 1) + J(1 - e^{-k_2^{\alpha} t})$$

where

$$F = k_2^{\alpha}k_1^{\alpha}/(\lambda_1 - \lambda_2)(k_2^{\alpha} + \lambda_1)$$

$$G = -k_2^{\alpha}k_1^{\alpha}/(\lambda_1 - \lambda_2)(k_2^{\alpha} + \lambda_1)$$

$$H = k_1^{\beta}(\lambda_4 + k_e + k_1^{\beta})/\lambda_3(\lambda_4 - \lambda_3)$$

$$I = -k_1^{\beta}(\lambda_3 + k_e + k_1^{\beta})/\lambda_4(\lambda_4 - \lambda_3)$$

$$J = -k_1^{\alpha}k_e[1/(k_2^{\alpha} + \lambda_1) - 1/(k_2^{\alpha} + \lambda_2)]/(\lambda_1 - \lambda_2)$$

and where the other parameters are as given above.

The parameters k_e and k_{-e} were eliminated from these equations, in order to achieve robust fits to the data, by expressing them in terms of experimentally determinable parameters, the burst ratios; that is, the ratios of **3** arising from **1aβ** to that from **2aα** (see main text) when either **1aα** (P_1) or **1aβ** (P_2) was the starting material. The expressions for k_e and k_{-e} below can be derived from the above equations by determination of the amount of **2aα** formed in each case. Thus

$$k_e = k_1^{\beta}P_2(1 + P_1)/(P_1 - P_2)$$

$$k_{-e} = k_1^{\alpha}(1 + P_2)/(P_1 - P_2)$$

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