Base-Catalyzed Hydrolysis of 6-Aminopenicillanic Acid. The Kinetic and Thermodynamic Products.

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In the presence of sodium hydroxide or a β-lactamase, 6-APA has been shown to hydrolyze rapidly at room temperature to penicic acid **3**, the kinetic product of the reaction. In a subsequent equilibration **3** isomerizes at C-5, by way of intermediate imine **4**, affording 5-*epi*-penicic acid **6** as the major hydrolysis product (~ 95% at equilibrium). The *p*H and temperature parameters of equilibration are discussed and HPLC, optical rotation, proton nmr and ^{13}C nmr data are presented.

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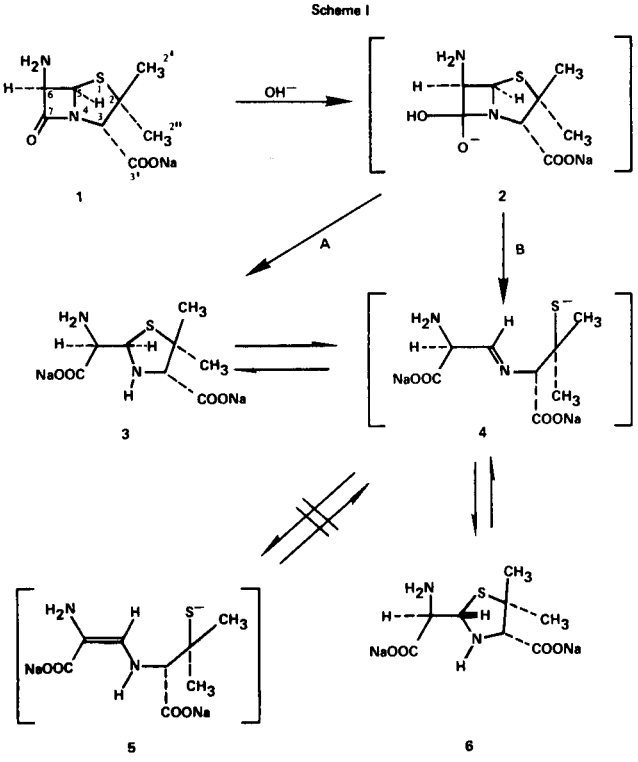
In connection with ongoing work we developed HPLC techniques for probing various penicillin reactions and their mechanisms. One of our systems was applied to the room temperature, alkaline hydrolysis of 6-aminopenicillanic acid (6-APA) **1** and revealed that a kinetic intermediate was formed which rearranged in high yield to the thermodynamically stable product. Previous studies (1a-e) reported that this product was penicic acid **3** but made no reference to the existence of a kinetic intermediate. Mechanistic considerations indicated that either pathway **A** or **B** (Scheme I), which differ only in how structure **2** rearranges, could explain our observations. Pathway **A** initially affords penicic acid **3** which then isomerizes at C-5, *via* imine **4**, to give 5-*epi*-penicic acid **6** as the thermodynamic, major product. Pathway **B** affords imine **4** as the kinetic product directly. Our work has shown that **1**, in fact, hydrolyzes according to Pathway **A** and thus the previous assignment of structure **3** to the major product of 6-APA hydrolysis is incorrect. We discuss herein the observations which support our conclusions.

Treatment of sodium 6-APA at room temperature with two equivalents of sodium hydroxide produced the rapid appearance of the kinetic intermediate which, in turn, equilibrated to an approximate 95:5 mixture of the thermodynamic and kinetic products respectively (HPLC monitoring, see Fig. 1). These same results were observed when **1** was reacted with a β-lactamase at 37° at *p*H7.0 (2). When the hydroxide hydrolysis of **1** was conducted at 0°, however, the kinetic product was found to be stable and could be kept in solution for nearly 24 hours by lowering the *p*H to 7 and allowing the solution to remain at room temperature. At elevated temperatures or at elevated *p*H, or both, the kinetic product slowly equilibrated to the thermodynamic mixture observed above. No attempt was made to isolate the kinetic product. The HPLC and nmr data were collected on material in solution. The thermodynamic product could be studied in solution as well, or isolated, if desired, by lyophilization.

The assignments of structures **3** and **6** to the kinetic and thermodynamic hydrolysis products, respectively, were made after examining the ^{13}C and ^{1}H nmr spectra of the two materials. The ^{13}C data revealed that the kinetic and thermodynamic products were similar yet distinctly different structures, each of which clearly

Scheme I



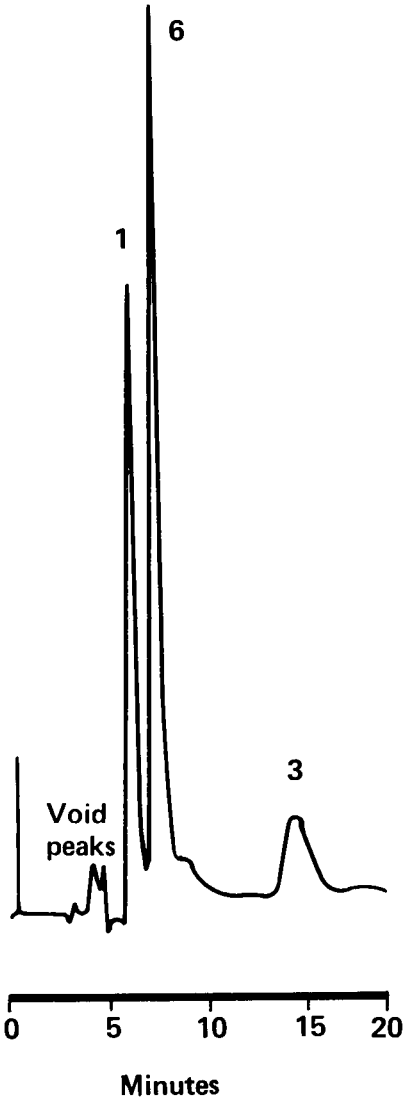


Fig. 1. A mixture of **1**, **3** and **6** illustrating resolution and retention times.

lacked a resonance corresponding to a trigonal, imino carbon atom (see experimental section). Thus imine **4** was excluded as a possibility for the kinetic intermediate. In the ^1H nmr spectra the kinetic product displayed a C-5*H*/C-6*H* coupling of 7 Hz whereas the thermodynamic species exhibited a 4 Hz coupling. This same trend (smaller coupling constant for 5-*epi* stereochemistry) was recently reported by Vanderhaeghe for the C-5 isomers of penicillin G penicilloic acid (3). Our results parallel his and indicate that likely all penicillins undergo epimerization at C-5 following base catalyzed hydrolysis (4). The preponderance of **6** at equilibrium is in accord with the findings of Stoodley which teach that thiazolidines substituted at C-2 and C-4 (C-3 and C-5 in the penicillin numbering system used here) favor *cis* substitutents (5).

That C-6 is not involved in the isomerization process was shown by carrying out the hydrolysis of **1** in deuterium oxide/sodium deuteroxide. Under these conditions no deuterium incorporation was observed. This finding excludes the intermediacy of enamine **5** which, *a priori*, might be expected to participate in the reaction and lead to isomerization at C-6. Finally, in the process **1**→**3**→**6** the respective optical rotations are 356°→311°→55°. The large change in going from **3** to **6** is consistent with an isomerization at C-5 but not C-6 (6).

We have used HPLC studies such as those described here to detect and elaborate several other penicillin reactions. We hope to publish our findings at a future date.

EXPERIMENTAL

HPLC experiments were performed on a DuPont Model 841 Liquid Chromatograph with 254 nm UV detector at 1000 psi (constant pressure) at ambient temperature using a 2.5 mm x 25 cm Micropak-Amine column (Varian Associates) with a mobile phase of 2.5% acetonitrile, 97.5% 0.0125 *M* potassium dihydrogen phosphate at *p*H 3.5. A typical chromatogram (of a partially equilibrated sample of **3** and **6**) which illustrates resolution and retention times is exhibited in Fig. 1. Samples to be analyzed were diluted with deionized water just prior to injection. Controls showed that the integrity of mixtures of **3** and **6** was not altered by contact with the *p*H 3.5 mobile phase.

The ^{13}C nmr spectra were obtained at *p*H 8.5 in the FFT mode on a Varian XL-100-15 (25 MHz) spectrometer equipped with a Nicolet Technology 1080 data system. Complete proton decoupling was provided by square wave modulation (7) of the Varian gyrocode heteronuclear decoupler. The spectra were obtained with a tip angle of 30° and an acquisition time of 1.4 seconds using quadrature phase detection. The field-frequency lock was maintained by solvent deuterium resonance (deuterium oxide) in a 5 mm (O.D.) sample tube. Dioxane (67.4 ppm) was used as the internal standard adjusted relative to TMS (8) to provide chemical shift values.

Proton nmr spectra were obtained on a Varian Model T-60 Spectrometer in deuterium oxide at *p*H 8.5 using DSS as internal standard. Optical rotations were obtained on a Perkin-Elmer Model 141 Polarimeter.

5-*Epi*-Penicic Acid (6).

A sample of 2.16 g. (10 mmoles) of 6-APA in 100 ml. of water at *p*H 7.5 was treated with 100 ml. (10 mmoles) of 0.100 *N* sodium hydroxide at room temperature for 140 hours. The *p*H ranged from 11.8 initially to 9.6 at the end of hydrolysis. The solution was lyophilized affording 2.68 g. (96%) of white noncrystalline **6**; ^1H nmr: δ 4.93 (d, J = 4 Hz, C-6*H*), 3.58 (d, J = 4 Hz, C-5*H*), 3.45 (s, C-3*H*), 1.60 and 1.26 ppm (2 s's, 2 x CH_3); ^{13}C nmr: δ 175.7 (C-3'), 173.4 (C-7), 75.4 (C-3), 67.4 (C-5), 60.5 (C-2 and C-6-coincident), 27.2 ppm (C-2' and C-2''-coincident). ^{13}C-Assignments were based on values obtained for 6-APA in deuterium oxide at *p*H 8.5 as compared to reported assignments for penicillin G (9). The product contained small quantities of 6-APA and **3**. Compound **6** could be prepared more conveniently by treating sodium 6-APA with 2 equivalents of sodium hydroxide at room temperature. Under these conditions hydrolysis was over

in 10-20 minutes. The *p*H remained >11 throughout the reaction and readjustment to 8.5 with 1 equivalent of hydrochloric acid, followed by lyophilization, afforded **6** free of 6-APA but contaminated with sodium chloride.

Penicic Acid (**3**).

A slurry of 2.5 g. (11.6 mmoles) of 6-APA in 5.5 ml. of deuterium oxide was cooled to 0° in a dry ice-acetone bath and 1.16 ml. of 40% sodium deuteroxide was added dropwise until a clear solution resulted (*p*H 7.5). At 0° 2.32 ml. of sodium deuteroxide was added all at once and after 10 minutes the *p*H was adjusted from 12.0 to 8.5 by the addition of deuterochloric acid. The solution was allowed to warm to room temperature for analysis. The HPLC trace obtained revealed rather pure **3** in solution contaminated with a small amount of **6** and residual 6-APA; ^{1}H nmr: δ 5.06 (d, J = 7 Hz, C-6*H*), 3.80 (d, J = 7 Hz, C-5*H*), 3.62 (s, C-3H), 1.75 and 1.33 ppm (2 s's, 2 x CH_3); ^{13}C nmr: δ 176.7 and 176.0 (C-3′ and C-7), 73.5 (C-3), 69.5 (C-5), 64.7 (C-2), 61.3 (C-6), 31.8 and 27.6 ppm (C-2′ and C-2″).

The reaction solution was monitored 20 hours after standing at room temperature at *p*H 8.5 and revealed essentially no change. However, if the solution was warmed to about 60° isomer **6** began to form slowly at the expense of **3** (see fig. 1). When the *p*H was raised at room temperature to 12 by the addition of sodium deuteroxide, **6** formed rapidly from **3**. Once equilibration was complete the 95:5 ratio of **6**:**3** was calculated from peak areas (see Fig. 1). The ^{1}H nmr spectrum also revealed that the proton at C-6 did not exchange during the reaction.

This experiment was repeated using a 6-APA concentration of 2.7 g./100 ml. and monitoring rotation. The initial specific rotation of 6-APA at *p*H 7.5 was 356°. The solution was cooled to 0° and treated with 2 equivalents of concentrated sodium hydroxide for 10 minutes, then readjusted to *p*H 7.5 with concentrated hydrochloric acid. The rotation dropped to 311° and remained unchanged at *p*H 7.5 at room temperature for over 2 hours. The *p*H was then adjusted to 12 with concentrated sodium hydroxide and, at room temperature, the rotation leveled off at 55° (readings were taken after adjusting the *p*H to 7.5 with hydrochloric acid) during a two hour period. The rotations are not corrected for the slight volume changes.

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