Communications to the Editor

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EPIMERIZATION OF BENZYLPENICILLOATE IN ALKALINE AQUEOUS SOLUTIONS

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5R,6R-Benzylpenicilloate (**2**) epimerized in alkaline aqueous solutions to 5S,6R-benzylpenicilloate (**5**) *via* the imine tautomer of benzylpenamaldate (**3**) rather than the enamine tautomer (**4**). In the presence of mercury(II) chloride, **2** isomerizes in alkaline aqueous solutions *via* **4** rather than **3** to afford **5**. In both cases, the 5S,6R-configuration was favored at equilibrium.

KEYWORDS———benzylpenicilloate; benzylpenicillin; benzylpenamaldate; benzylpenicilloate epimerization

It is well known that in the presence of alkali or a β-lactamase, penicillins are hydrolyzed to the corresponding penicilloates.[1] Studies[2] of the epimerization of the penicilloates on storage in aqueous solutions have shown that a 5R,6R-penicilloate, which is formed by hydrolysis of a penicillin, slowly epimerizes in aqueous solutions to a 5S,6R-penicilloate, and the latter is preferred at equilibrium. On the other hand, from only ultraviolet spectral evidence it is thought that a penicilloate is converted to the enamine tautomer of a penamaldate in the presence of mercury(II) chloride.[3] While the present work was in progress, a similar study on epimerization of benzylpenicilloate in weakly alkaline aqueous solutions was published.[4] The aim of this communication is to clarify the mechanism of epimerization of benzylpenicilloate in alkaline aqueous solutions in the absence or the presence of mercury(II) chloride.

Figures 1a and 1b show the time-dependent nuclear magnetic resonance (NMR) spectral changes of degradation of benzylpenicillin (**1**) in alkaline aqueous solutions in the absence and the presence of mercury(II) chloride. The chemical shifts and assignments of the signals are given in Table I. The signal assignments were made taking into account the previously reported chemical shifts and coupling constants of 5R,6R- (**2**) and 5S,6R-benzylpenicilloate (**5**),[4] and the dimethylester of two epimers.[2b] There are two differences in the NMR spectra of Figs. 1a and 1b: one is the loss of the 6-H doublet signal of **5** in Fig. 1b, and the other is the replacement of the 5-H doublet signal of **5** in Fig. 1a with the broad singlet signal of **5** in Fig. 1b. These differences are due to the deuteration of the 6-H proton of **5** in alkaline aqueous solutions in the presence of mercury(II) chloride. Thus, the results reveal that **1** is rapidly degraded in alkaline aqueous solutions (pD 13.0) to yield **2**, which slowly epimerizes to **5** in the presence as well as in the absence of mercury(II) chloride.

High-performance liquid chromatography (HPLC) of the alkaline degradation products of **1** showed two peaks at retention times of about 4 and 6 min independent of the absence or the presence of mercury(II) chloride. As the reaction proceeded,

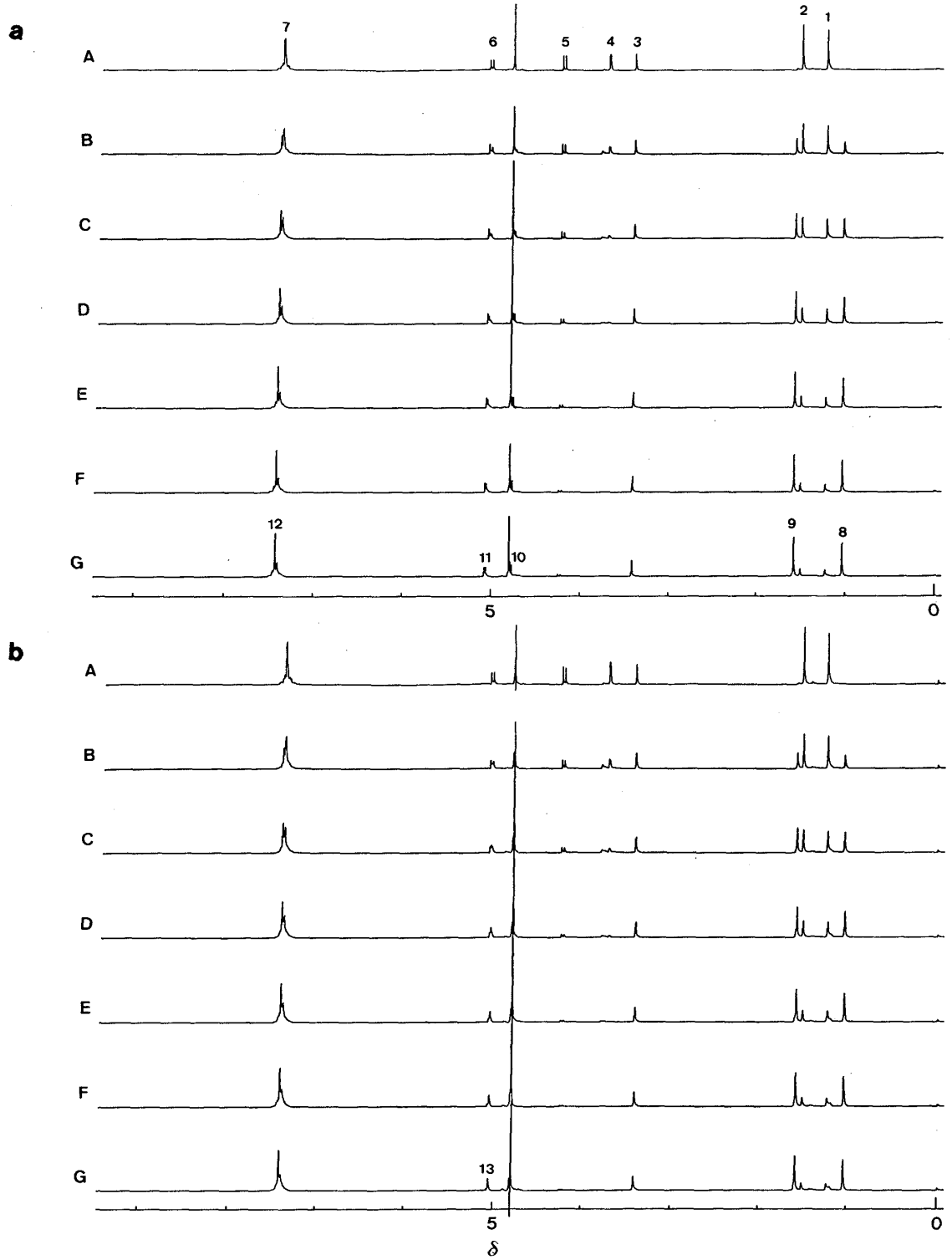

Fig. 1. ^{1}H-NMR Spectra of Benzylpenicillin Degraded in Alkaline Aqueous Solutions in the Absence (a) and the Presence (b) of Mercury(II) Chloride at Specific Time Intervals: A, 0.2 h; B, 4 h; C, 8 h; D, 12 h; E, 16 h; F, 20 h; G, 24 h. Potassium benzylpenicillin (10 mg) was dissolved in 1 ml of 0.1 M NaOD plus 0.15 M sodium carbonate in the absence (a) and the presence (b) of 5×10^{-4} M mercury(II) chloride.

Table I. ^{1}H Chemical Shifts of Benzylpenicilloates

Line number[a]	5R,6R-Epimer	5S,6R-Epimer
1	1.22(CH_3)	
2	1.50(CH_3)	
3	3.41(3-H)	3.41(3-H)
4	3.70(10-H)	
	3.72(10-H)	
5	4.22(5-H)[b]	
	4.25(5-H)	
6	5.04(6-H)[b]	
	5.07(6-H)	
7	7.4 (C_6H_5)	
8		1.04(CH_3)
9		1.57(CH_3)
10		4.78(5-H)[c]
		4.79(5-H)
11		5.06(6-H)[c]
		5.07(6-H)
12		7.4 (C_6H_5)
13		5.06(6-H)

a) Line numbers refer to Fig. 1. b) $J_{5,6}$=5.9 Hz.
c) $J_{5,6}$=3.1 Hz.

the peak at the retention time of about 6 min (**2**) decreased with a concomitant increase of that of about 4 min (**5**). The time-dependent changes of the peak area ratio of the two peaks were similar to those obtained by NMR spectra. Thus, these HPLC results confirm that **2** isomerizes in alkaline aqueous solutions in the presence as well as in the absence of mercury(II) chloride to afford **5**.

Based on mechanistic considerations, two pathways (Chart 1) are proposed for epimerization of benzylpenicilloate. The first is pathway A which passes through the imine tautomer of benzylpenamaldate (**3**) as an intermediate. The second is pathway B which passes through the enamine tautomer of benzylpenamaldate (**4**) as an intermediate. Ghebre-Sellassie and his co-workers[4] recently reported that **2** isomerized in alkaline aqueous solutions *via* **3** (Chart 1, pathway A) to give **5**. The NMR results described above suggest that **2** isomerizes in alkaline aqueous solutions in the presence of mercury(II) chloride *via* **4** (Chart 1, pathway B) to give **5**. However, the NMR signals due to benzylpenamaldate[2c] were not noticeable in the epimerization process. It is concluded that in the absence of mercury(II) chloride, **2**, whose configuration is the same as that of **1**, epimerizes in alkaline aqueous solutions to **5** *via* **3** rather than **4**; on the other hand, in the presence of mercury(II) chloride, the epimerization takes place *via* **4** rather than **3**.

The kinetics of mercury(II)-catalyzed epimerization and the role of mercury(II) ion in epimerization are now being investigated.

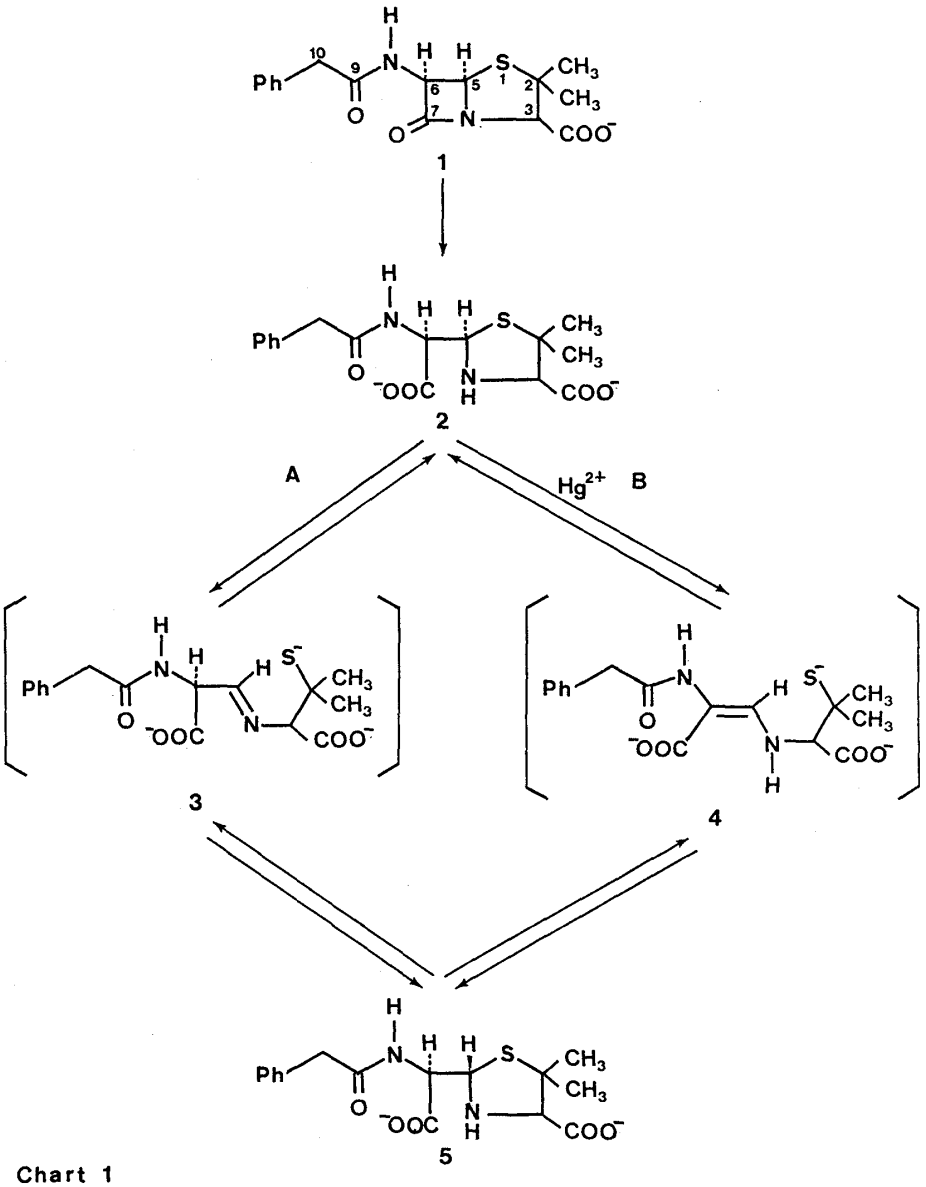

Chart 1

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