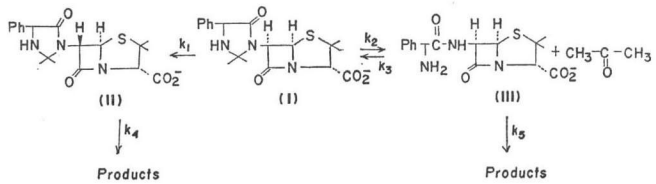
KINETIC STUDY ON EPIMERIZATION AND HYDROLYSIS OF HETACILLIN: USE OF NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Sir:

In the degradation of hetacillin (**I**) in aqueous solution, there are at least two possible reactions: the epimerization to 6-epihetacillin (**II**) and the interconversion of **I** to ampicillin (**III**). The overall reaction pathways are illustrated in Scheme 1.[1] The rates of both hydrolysis and epimerization are pH dependent and occur simultaneously in competing reactions. Studies on the epimerization to **II**[2-4] and the conversion to **III**[1,5-8] have been reported by several workers, but little is known about the epimerization rates. Because of the clinical significance of epiampicillin (**IV**) produced from **II** having little antibacterial potency,[3] it is important to determine the relative rates of k_1 and k_2 at a desired pH value.

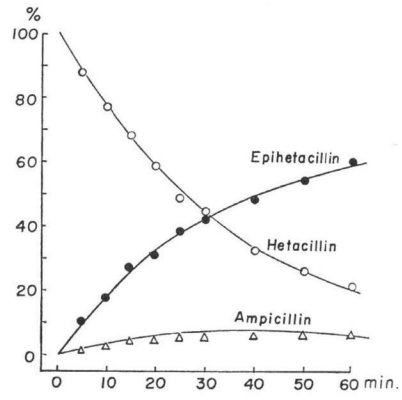
Scheme 1.



The purposes of this investigation were to develop a method for quantitating epimerization by use of n.m.r. spectroscopy and to determine the rates of hydrolysis and epimerization of **I** in D_2O solution.

In an n.m.r. spectrum* of a mixture of **I**, **II** and **III**, the chemical shifts for the C-3 protons in the region of δ 4.2~4.4 [**I**, 4.30; **II**, 4.39; **III**, 4.19] differ sufficiently to determine the relative amounts of these antibiotics and other degradation products. In this work, the amounts of **I**, **II** and **III** were calculated at various time intervals from the integral value for each C-3 proton and the total integral value for five phenyl protons in the region between δ 7.39 and 7.51. Fig. 1 gives the data for the reaction of **I** at pD 10.6 and 35°C, and shows that the amounts of **II** and **III** increase to 60% and 6% respectively after 1 hour. The completed curves were simulated on an analog computer which was also used to generate the rate constants (in h^{-1}); k_1=1.30, k_2=0.30, k_3=0.0, k_4=0.11 and k_5=1.20. The rate costants, k_4 and k_5, were determined from the degradation of **II** and **III**, respectively. The reverse reaction from **III** and acetone to **I** is negligible under these conditions because of a small k_3[1] and low concentration of **III** and acetone. In this spectrum at pD 10.6, the two signals at δ 3.30 and 3.52 assigned to the C-3 protons in the β-lactam opening products of **III** and **IV** had appeared, but there were no apparent signals corresponding to those for intact **IV** and β-lactam opening products of **I** and **II**. These results suggest that the β-lactams of **I** and **II** are considerably stablized by the steric

Fig. 1. Time courses for hetacillin, epihetacillin and ampicillin during the degradation of hetacillin in Na_2CO_3-$NaDCO_3$ buffer of pD 10.6 at 35°C.

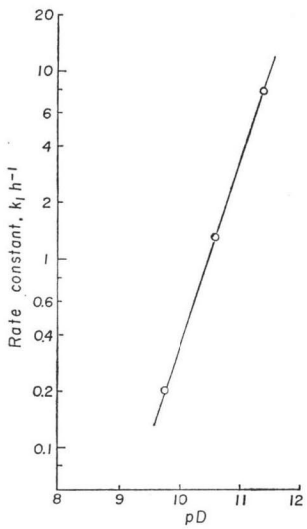


* The spectra were obtained at 35°C in D_2O solution on a JEOL JNM-PS-100 spectrometer operating in a field sweep mode. Sodium 2,2,3,3-tetradeutero-3-(trimethylsilyl) propionate was used as internal standard. The spectra were recorded on solutions at concentration of 40 mg /ml.

hindrance of the *gem*-dimethyl groups of the imidazolidin ring toward hydroxide-ion attack, whereas that of **III** and **IV** can be easily hydrolyzed under these conditions.

At pD 11.4 I epimerized (k_1=8.0 h^{-1}) almost completely to **II** after 40 minutes, whereas at pD 6.0 **I** converted (k_2=1.6 h^{-1}) completely to **III** after 3 hours at 35°C. At pD 10.6 and 9.75 the degradation of **I** produced both **II** and **III**. The reaction of **II** at pD 9.75, 10.6 and 11.4 gave no detectable amount of **I**, which support the previous report[8] of the irreversible conversion of **I** into **II**. A plot of log k_1 against pD yielded a straight line with a possitive slope, verifying a first-order dependence on hydroxide ion. The log k_1-pD profile is illustrated in Fig. 2.

Fig. 2. Log k_1 *vs* pD for the epimerization of hetacillin at 35°C and μ=0.5.



The magnitude of the epimerization rate constants together with our previous observations[1] lead to the conclusion that epimerization represents a major pathway of degradation and inactivation of hetacillin at high pH and that the reaction for the formation of biologically active antibiotic **III** is predominant in the neutral pH region.

The detailed kinetics of the epimerization are being investigated also by ORD measurement in H_2O. Results of these studies will be reported in more complete paper.

Acknowledgements

We thank Banyu Pharm. Co., Ltd. for the gift of potassium hetacillin and Takeda Chemical Ind. Ltd. for sodium ampicillin.

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(Received September 24, 1974)

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