

## Intramolecular Nucleophilic Attack in 6-Epi-ampicillin

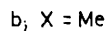
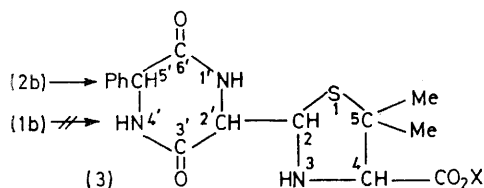
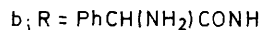
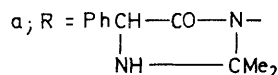
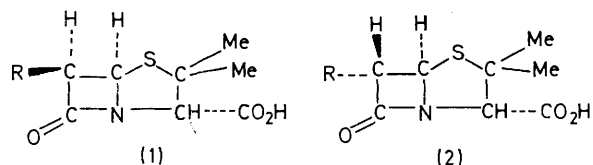
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*Summary* 6-Epi-hetacillin slowly hydrolyses in neutral aqueous solution to 6-epi-ampicillin, which then gradually cyclizes to 2-(3,6-dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid by intramolecular nucleophilic attack of the side-chain amino-group upon the  $\beta$ -lactam carbonyl; under the same conditions hetacillin readily hydrolyses to yield ampicillin as sole product.

HETACILLIN (**1a**) has been transformed into 6-epi-hetacillin (**2a**) by treatment with aqueous alkali.<sup>1</sup> As we needed 6-epi-ampicillin (**2b**) as an intermediate for the preparation of 6-epi-APA (**2c**), we examined the hydrolysis of 6-epi-hetacillin (**2a**) in various solvents and under different conditions.

In all our experiments 6-epi-hetacillin (**2a**) was hydrolysed much more slowly than hetacillin (**1a**) and the reaction mixture contained not only 6-epi-ampicillin (**2b**) plus starting material, but also a side product, which was not formed in the hydrolysis of hetacillin (**1a**). The highest yield (64%) of 6-epi-ampicillin (**2b**) was obtained after hydrolysis in neutral aqueous solution for several hours at room temperature.<sup>2</sup> However, pure side-product (52% yield) was obtained after reaction for 5 days. The yield was improved (up to 72%) by adding pyridine-AcOH. Structure (**3a**) was assigned to this compound, m.p. 189—191° (decomp.),  $[\alpha]_D^{20} + 222^\circ$  (*c* 0.5 in 0.5M NaHCO<sub>3</sub>);  $\nu_{\max}$ . (KBr) 3340—3300, 1680—1660, 1450, 1330—1290

(amide), 3200, 1130 (amine), and 1720—1700 (CO<sub>2</sub>H) cm<sup>-1</sup>,  $\delta$  [(CD<sub>3</sub>)<sub>2</sub>SO; hexamethyldisilane) 1.14 and 1.37 (s, *gem*-Me<sub>2</sub>), 3.62 (s, 4-H), 4.19 (m, 2'-H), 4.96 (m, 5'-H), 5.17 (d, *J* 4 Hz,



2-H), 7.22—7.68 (m, Ph), 8.08 (d, *J* 2.5 Hz, 1'-H), and 8.53 (d, *J* 2 Hz, 4'-H). On addition of D<sub>2</sub>O the amide and amine protons disappear, and the multiplets at 4.19 and 4.96 collapse to doublets with *J* 4 and 1 Hz respectively. The acid was transformed into the methyl ester (**3b**) m.p. 218—220° (decomp.),  $[\alpha]_D^{20} + 162^\circ$  (*c* 0.5 in pyridine); *m/e* 363. I.r. and n.m.r. data confirmed the structure proposed for the acid. Compound (**3a**) shows a spectrophotofluorimetric excitation maximum at 363 nm and emission maximum at 453 nm (uncorrected). These values correspond well with those observed by Jusko<sup>3</sup> for the product obtained by heating ampicillin (**1b**) in acid solution and which is assumed to be a 2,5-substituted diketopiperazine derivative.

The formation of the diketopiperazine (**3a**) from 6-epi-ampicillin (**2b**) in neutral aqueous solution is due to nucleophilic attack of the amino-group of the side-chain upon the  $\beta$ -lactam carbonyl from the unhindered *exo*-side of the molecule. The stability of ampicillin (**1b**) under the same conditions can be explained by the fact that the same reaction must occur from the *endo*-side, where considerable steric hindrance operates. This is consistent with the recent report<sup>4</sup> of the conversion of cephalosporin derivatives into 2,5-diketopiperazines in refluxing benzene, while ampicillin trichloroethyl ester remains unchanged under the same conditions.

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<sup>1</sup> D. A. Johnson, D. Mania, C. A. Panetta, and H. H. Silvestri, *Tetrahedron Letters*, 1968, 1903.

<sup>2</sup> J. P. Clayton, J. H. C. Naylor, R. Southgate, and E. R. Stove, *Chem. Comm.*, 1969, 129.

<sup>3</sup> W. J. Jusko, *J. Pharm. Sci.*, 1971, **60**, 728.

<sup>4</sup> J. M. Indelicato, T. T. Norvilas, and W. J. Wheeler, *J.C.S. Chem. Comm.*, 1972, 1162.