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 β-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.[1] 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.[2] 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° (*c* 0·5 in 0·5M $NaHCO_3$); $\nu_{max.}$ (KBr) 3340—3300, 1680—1660, 1450, 1330—1290

(amide), 3200, 1130 (amine), and 1720—1700 (CO_2H) cm^{-1}, δ [$(CD_3)_2SO$; hexamethyldisilane) 1·14 and 1·37 (s, *gem*-Me_2), 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_2O 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° (*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[3] 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 β-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[4] 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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