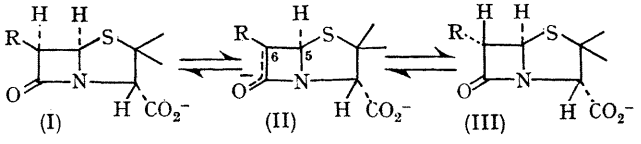
Penicillanic Acids: Requirements for Epimerisation at C-6

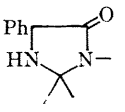
By J. P. CLAYTON, J. H. C. NAYLER,* R. SOUTHGATE, and E. R. STOVE
(*Chemistry Department, Beecham Research Laboratories, Brockham Park, Betchworth, Surrey*)

6-BROMO- AND 6-CHLOROPENICILLANIC acids have been prepared by the diazotisation of 6(β)-aminopenicillanic acid (Ia),[1] and the chloro-compound has been shown to have structure (IIIb), resulting from inversion at C-6.[2] Similarly we have found that the 6-bromo-compound has structure (IIIc), $J_{5,6}$ 1·5 Hz.[2] In an attempt to equilibrate 6(α)-bromopenicillanic acid (IIIc) with its 6(β)-epimer (Ic), (IIIc) was dissolved in NaOH at pH 10—11 and the solution acidified after 5 hr., but the recovered acid was shown by n.m.r. to be unchanged (IIIc). However, when (IIIc) was similarly treated in D_2O–NaOD, it was observed that the doublet at δ 4·82 (6-H) slowly disappeared while the doublet at δ 5·41 (5-H) sharpened to a singlet. The deuteriated acid was liberated with DCl and isolated as an oil, which after treatment with diazomethane furnished the crystalline methyl ester, m.p. 46—48°, the mass spectrum of which confirmed the incorporation of one atom of deuterium. We conclude therefore that the C-6 proton in (IIIc) is sufficiently acidic to permit formation of anion (IIc), but that the latter reprotonates exclusively from the *endo*-side. This is consistent with the recent report[3] of the irreversible conversion of hetacillin (Id) into epihetacillin (IIId) by means of aqueous alkali.

We investigated anion formation at C-6, and its possible application in the preparation of novel epimers, by examining the behaviour of various penicillanic acids in aqueous base. 6(β)-Amino-penicillanic acid (Ia), its *NN*-dimethyl derivative[4] (Ie), and 6(β)-tritylaminopenicillanic acid[5] (If) during 30 min. at pH 10–11 gave no indication of epimerisation. Similarly in deuteriated base, penicillanic acid[6] (Ig), showed no evidence of deuterium incorporation. More vigorous treatment of these acids resulted in rupture of the sensitive β-lactam ring. It thus appears that the prime factor in determining whether anion formation will occur is the electronegativity of the 6-substituent. Various penicillins (I; R = acylamino) all failed to epimerise at pH 11, which is at first sight surprising in view of the ready epimerisation of hetacillin. In the case of the penicillins we suggest that the first proton to be removed by base may be that of the secondary amide group, and that the proximity of the resulting negative charge then prevents the loss of a second proton from C-6.



a: R=NH_2; b: R=Cl; c: R=Br d: R=



e: R=NMe_2; f: R=$NHCPh_3$ g: R=H; h: R=$\overset{+}{N}Me_3$,

j: R=$PhCH(NH_3)\cdot CO\cdot NH-$; k: R=$PhCH(NH\cdot CH_2Ph)\cdot CO\cdot NH-$

A trimethylammonium substituent at C-6 would be expected to facilitate proton removal. The betaine (Ih), in the form of the highly crystalline hemihydroiodide, was first obtained by Leigh[4] from 6(β)-dimethylaminopenicillanic acid and methyl iodide. The same salt is still more readily prepared by adding a large excess of methyl iodide to lithium 6(β)-aminopenicillanate dissolved in methanol, crystallisation of the hemihydroiodide commencing after a few minutes; m.p. 189° (decomp.), $[\alpha]_D^{25}$ = 212° (*c* 0·3%, water), n.m.r. (D_2O), δ 5·47 (d, 5-H), 5·80 (d, 6-H, $J_{5,6}$ 4 Hz). With a view to liberating the free betaine, this salt was dissolved in water and brought to pH 7 with sodium bicarbonate. The solution was lyophilised and the residue

crystallised from aqueous acetone to give a product,[7] m.p. 171°, $[\alpha]_D^{20}$ + 207° (*c* 0·3%, water) which was shown by n.m.r. to be the epibetaine (IIIh), n.m.r. [$(CD_3)_2SO$], δ 6·03 (d, 5-H) and 5·23 (d, 6H, $J_{5,6}$ 1·5 Hz). Addition of D_2O to the $(CD_3)_2SO$ solution, or dissolution of (IIIh) in D_2O, resulted in rapid replacement of the C-6 proton by deuterium. Treatment of (IIIh) with hydroiodic acid afforded the crystalline epi-betaine full hydroiodide,† m.p. 180°, $[\alpha]_D^{25}$ + 151° (*c* 0·3%, water).

When the hemihydroiodides‡ of (Ih) and (IIIh) were each kept in D_2O buffered at pH 5·9 and the n.m.r. of the two solutions examined after 45 min., it was found that 86% replacement of the C-6 proton had occurred in the case of (Ih), but only 12% replacement in the case of (IIIh). Conversion of (Ih) or (IIIh) into deuteriated (IIIh) was rapid and complete above pH 7, but was not observed at all below pH 4.

In a further experiment hetacillin (Id) and epihetacillin[3] (IIId) were each dissolved in D_2O–NaOD at pH 9·5 and after 30 minutes the solution was acidified with DCl and the precipitated solid (70% recovery) was collected and examined by n.m.r. The product from (Id) contained about 60% of epimer (IIId) deuteriated at C-6, the remaining 40% being undeuteriated (Id). The product from (IIId) contained approximately 30% deuterium at C-6 but no detectable amount of epimer (Id).

The observations on relative rates of deuterium incorporation suggest that the ionisation step is faster in the direction (I → II) than (III → II), but the difference seems insufficient to account for the observed overwhelming preponderance of the trans epimer (III) at equilibrium. We conclude that the structure of the product is mainly determined at the reprotonation step. Formation of (III) rather than (I) may be attributed to a lower energy barrier between (II) and (III) than between (II) and (I), where the transition state would involve appreciable steric compression between R and the nearer of the geminal methyl groups.

Methyl 6(β)-phthalimidopenicillanate undergoes C-6 epimerisation in the presence of anhydrous base.[7] The β-elimination mechanism originally suggested involved fission of the thiazolidine ring, but our simpler mechanism seems adequate for aqueous systems.

Finally the epimerisation of hetacillin provided an indirect route to the epimer of the important broad-spectrum antibiotic ampicillin (Ij). Both hetacillin and epihetacillin readily hydrolyse in neutral aqueous solution with the loss of acetone.

A solution of epihetacillin (IIId) was kept at pH 7 for 3 hr., acidified, filtered to remove undissociated (IIId) (~50%), and the filtrate lyophilised. The residual epi-ampicillin (IIIj) had no more than 2% of the antibacterial activity of ampicillin, and contamination with the latter may have been responsible for even this low order of activity. Epiampicillin was characterised by reaction with benzylbromide in methylene chloride-triethylamine to give the benzyl ester of (IIIk),† m.p. 63—65°, $[\alpha]_D^{21}$ + 105° (*c* 1·1, $CHCl_3$).

We thank Dr. R. J. Stoodley for a stimulating discussion concerning the reaction mechanism and Mr. K. W. B. Austin and Mr. R. Edser for n.m.r. spectra.

(*Received, October 28th*, 1968; *Com.* 1452.)

† Elemental analyses and i.r. and n.m.r. spectra were satisfactory.

‡ The epi-betaine hemihydroiodide was prepared by mixing equimolar amounts of (IIIh) and its full hydroiodide.

[1] G. Cignarella, G. Pifferi, and E. Testa, *J. Org. Chem.*, 1962, **27**, 2668.
[2] I. McMillan and R. J. Stoodley, *Tetrahedron Letters*, 1966, 1205.
[3] D. A. Johnson, D. Mania, C. A. Panetta, and H. H. Silvestri, *Tetrahedron Letters*, 1968, 1903.
[4] T. Leigh, *J. Chem. Soc.*, 1965, **3616**.
[5] S. Wolfe, *Canad. J. Chem.*, 1968, **46**, 459.
[6] E. Evard, M. Claesen, and H. Vanderhaeghe, *Nature*, 1964, **201**, 1124.
[7] S. Wolfe and W. S. Lee, *Chem. Comm.*, 1968, 242.