A Ready C-6 Epimerization of the Penicillin Nucleus

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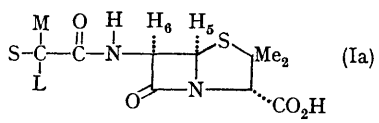
ALTHOUGH the precise fate of penicillin at its site of action is still uncertain, it is known that some of the antibiotic becomes covalently bonded to a substance present in growing bacterial cells[1]. The suggestion has been made[2] that the active site of an enzyme needed for the construction of the cell wall is occupied by penicillin and then acylated by the β-lactam. More recently the biological process which is blocked by penicillin has been found to be a transpeptidation reaction in which a D-alanine unit is removed from a *N*-acyl-L-lys-D-ala-D-ala-CO_2H peptide.[3] Strominger[3a] has suggested that his results imply the existence of a structural relationship between penicillin and this peptide. The L-lys-D-ala peptide bond would then correspond to the amide side-chain of penicillin, and the D-ala-D-ala peptide bond to the β-lactam.

There are two obvious difficulties with this picture: the absolute configuration of penicillin at C-6 is L-rather than D-; and D-α-amino-acid derivatives of 6-APA appear to be more active than their epimers.[4] These difficulties may not be severe, however, since the relative antibacterial activities of diastereomeric penicillins [*e.g.* (Ia)] can be rationalized in conformational rather than configurational terms.[5]

We now report results which seem relevant to the above discussion. Epimerization (L- to D-) at C-6 of methyl 6-phthalimidopenicillanate can be effected readily by a variety of reagents.[6] Although the mechanism of the epimerization is not yet certain we believe that a simple deprotonation–reprotonation does not occur.

Treatment of methyl 6-phthalimidopenicillanate,[7] m.p. 175°, $[\alpha]_D$ +279° (chloroform) with sodium hydride–tetrahydrofuran (A), potassium t-butoxide–t-butanol (B), or triethylamine–CH_2Cl_2



(Ia)

(C) afforded not the expected anhydropenicillin[8] but, in each case, in over 50% yield, an isomer of the starting material, m.p. 183°, $[\alpha]_D$ +228° (chloroform). The infrared spectra of the two compounds were very similar as were the n.m.r. spectra from which, however, the nature of the reaction was readily deduced. The spectrum of the starting material shows β-lactam absorption at τ 4·33 [H(6); *J* 4·2] and 4·40 [H(5); *J* 4·2 Hz]. The product shows β-lactam absorption at τ 4·40 (1H, *J* 2·0 Hz) and 4·62 (1H, *J* 2·0 Hz). Since a coupling constant of 2·0—2·5 Hz corresponds to a *trans*-arrangement of the β-lactam protons,[9] the isomer has the epimeric configuration at C(5) or C(6). Procedure A and work-up with D_2O produced epimer containing no deuterium. Procedure B in t-BuOD produced epimer containing

one deuterium, the absorption at τ 4·62 being absent from the n.m.r. spectrum.

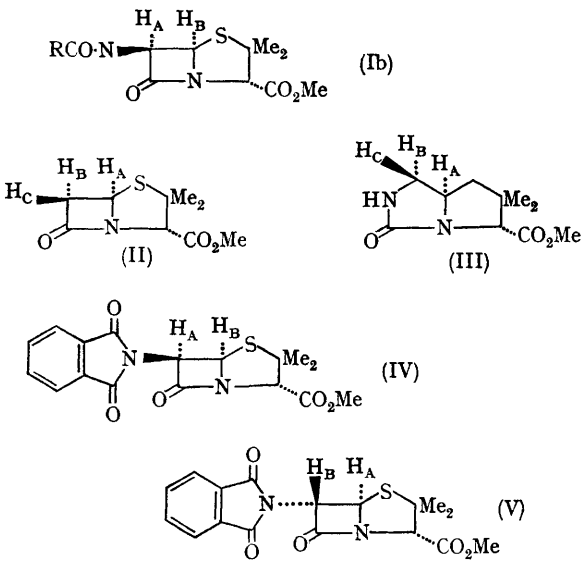
This means that, if deuterium is attached to C-6, the *cis* → *trans*-conversion must be accompanied by a reversal of the chemical shifts of H(5) and H(6). That this is the case is evident from the data shown in the Table. In compounds

TABLE

Chemical shift data for some penicillin derivatives

Compound	H_A	H_B	$H_C(\tau)$
(Ib)	4·33	4·45	
(II)*	4·71	6·42	6·95
(III)	4·33	6·08	6·42
(IV)	4·33	4·40	
(V)	4·40	4·62	

* H_A and H_B are taken to have the *alpha*, and H_C the *beta* configuration.



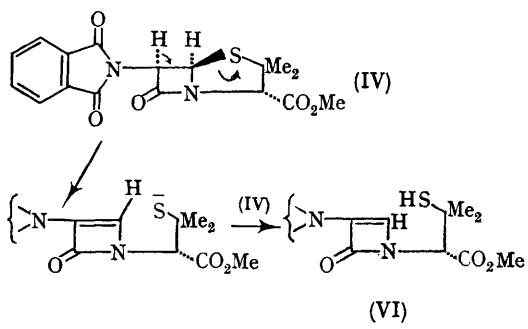
(II) and (III), β-H(6) is at 0·53 and 0·34 p.p.m. higher field, respectively, than α-H(6). The structure shown for (V) is assigned on the basis that H(5) has the same chemical shift in (IV) and (V) and β-H(6) (compound V) is at 0·29 p.p.m. higher field than α-H(6) (compound IV).

Recovered (IV) from procedure B (t-BuOD) contained no deuterium. Under the same conditions, (V) was recovered quantitatively and, significantly, there was no incorporation of deuterium. These observations indicate that the (IV) → (V) epimerization is irreversible (an unexpected result for an acid–base reaction) or that in a scheme

$$\text{(IV)} \underset{k_{-c}}{\overset{k_c}{\rightleftharpoons}} \text{"anion"} \underset{k_t}{\overset{k_{-t}}{\rightleftharpoons}} \text{(V)},$$

$k_{-t} \gg k_{-c}$ and $k_{-t} \gg k_t$. It is not unusual that equilibrium should favour (V) since epimerization changes the configuration from *endo*- to *exo*-, but it does seem surprising that k_{-t} should be much greater than k_{-c}, even when proton transfer to the *endo* face of "anion" is from the very bulky species (IV) (conditions A).

These results have led us to consider alternate routes for the epimerization and, in particular, the β-elimination shown (analogous to the first step of the anhydropenicillin rearrangement):



Interestingly, such a fragmentation, followed by S–S bond formation at the active site, is also consistent with the known facts concerning the mode of the action of penicillin. Attempts to isolate or trap (VI) have so far been unsuccessful. Treatment of (IV) under conditions A or B in the presence of methyl iodide, benzyl chloride, benzyl bromide, *p*-nitrobenzyl bromide, methyl acrylate, phenoxyepoxypropane, or acetonitrile gave no product of either S-trapping or C-trapping. Indeed, none of these compounds had any effect upon the (IV) → (V) epimerization. The unsaturated thiol (VI) is an enamine and might be expected to undergo partial loss of the side-chain during the workup; in agreement with this, *phthalimide* can be isolated from the epimerization of (IV) and

tritylamine can be isolated when methyl 6-tritylaminopenicillanate is treated with t-BuOK–t-BuOH. The evidence in support of a β-elimination pathway for epimerization is thus permissive but not yet compelling.

(*Received, December 18th,* 1967; *Com.* 1347.)

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