Thiazolidine Ring Opening in Penicillin Derivatives. Part 1. Imine Formation

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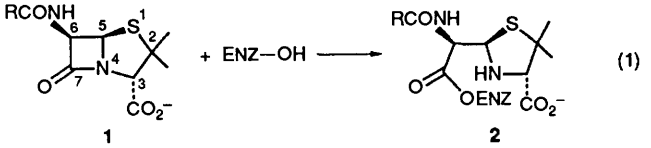
The rate of epimerisation of (3*S*,5*R*,6*R*)-benzylpenicilloic acid at C-5 shows three distinct dependencies upon pH in aqueous solution. Below pH 6 the rate shows a sigmoidal dependence upon pH, whereas it is pH-independent between pH 6 and 12, and above pH 12 the rate is hydroxide-ion dependent. These different regions of pH dependence are interpreted in terms of three mechanistic pathways all of which involve opening the thiazolidine ring by C–S bond fission and re-closure to generate the epimer. At low pH the reaction occurs by unimolecular ring opening of the *S*-conjugate acid which is kinetically equivalent to the *N*-conjugate acid of pK_a 5.14. The pH-independent pathway involves formation of a zwitterion by unimolecular opening of the neutral thiazolidine. At high pH the unprotonated imine intermediate is formed by concerted hydroxide-ion-catalysed ring opening. The mono- and di-methyl esters of benzylpenicilloate also epimerise at C-5. At low pH the rates are similar for all three compounds but above pH 6 the mono- and di-esters are, respectively, 21 and 1700 times less reactive than the dianion of the diacid.

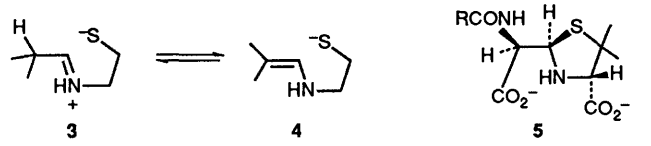
The first chemical step in the antibacterial activity of penicillins **1** is thought to be the opening of the β-lactam ring by a serine hydroxy group of a transpeptidase enzyme to form a penicilloyl enzyme intermediate **2**, which is an ester of penicilloic acid [eqn. (1)].[1–3] It is not known if it is the resistance of this intermediate



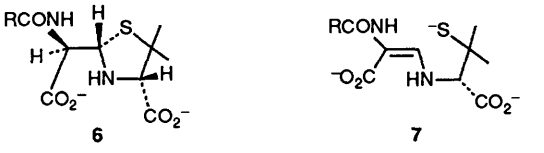
(1)

towards hydrolysis and the consequential lack of regeneration of the enzyme which causes the inhibition. It is conceivable that a reaction of the penicilloyl ester produces an electrophilic entity which is ultimately responsible for enzyme inhibition by irreversibly reacting with another nucleophilic group on the enzyme.[1]

Thiazolidines undergo hydrolysis to the aldehyde and amino thiol which is thought to proceed by the intermediate formation of an iminium thiolate zwitterion **3**.[4–6] In addition to undergoing nucleophilic attack at the iminium ion carbon the zwitterion can rearrange to the corresponding enamine **4**.[6] In fact, it has been suggested that elimination across C_5–C_6 of penicillin derivatives occurs directly to give the enamine **7** which can be stabilised by mercury(II).[7]



Convenient analytical tools for examining thiazolidine ring opening and elimination across C_5–C_6 in penicillin derivatives are polarimetry, NMR, deuterium exchange, chromatography and UV absorption spectrometry. Natural penicillins, **1**, have a 5*R*,6*R* configuration and there have been several studies reporting a variety of conditions which give rise to a change in one or both of these configurations.[1,8] Hydrolysis of penicillin gives penicilloic acid, **5**, which slowly epimerises to the 5*S*,6*R* configuration, **6**,[9–11] and it has been suggested that the



penicilloic acids are in equilibrium with the enamine, penamaldic acid (**7**).[12] Epimerisation at C-6 in penicillins appears to occur with and without deuterium exchange and both processes may occur at the enamine **7** or at the carbanion level.[1]

We report here the pH dependence of the rate of epimerisation at C-5 of (3*S*,5*R*,6*R*)-benzylpenicilloic acid (**5**, R = $PhCH_2$) and its mono- and di-methyl esters **8** and **9**. In the following paper, the epimerisation at C-6 and C-5 and thiazolidine ring opening reactions of penicilloyl esters **8** and **9** and amides are described. A preliminary description of this work has been published.[1,13]

Experimental

(3S,5R,6R)-*Methyl Benzylpenicilloate* (**8**).—Benzylpenicillin (6 g) was dissolved in methanol (100 cm^3) and triethylamine (4 cm^3) and kept at room temperature, under nitrogen, for 72 h. The solvent was removed under reduced pressure to give a residue which was taken up in water (75 cm^3) and diethyl ether (75 cm^3) and then acidified to pH 3.5 with 1 mol dm^{-3} hydrochloric acid. The diethyl ether layer was separated and the aqueous phase further extracted with diethyl ether. The combined fractions were dried and evaporated to give a white solid which was recrystallised from methanol–diethyl ether (1:9). An alternative method took benzylpenicillin (2.0 g) in methanol (10 cm^3) to which was added a catalytic amount of 1 mol dm^{-3} sodium hydroxide (50 mm^3). This gave a much higher yield (90%). M.p. 125–127 °C (lit.,[14] 125.5–127 °C).

(3S,5R,6R)-*Dimethyl Benzylpenicilloate* (**9**).—This compound was prepared according to the method described by Busson.[14]

^{1}H *NMR Studies.*—(*i*) Benzylpenicillin (50 mg) was dissolved in 0.1 mol dm^{-3} NaOH (25 cm^3) and left for four days at room temperature, then freeze-dried and redissolved in D_2O. (*ii*) Benzylpenicillin (20 mg) was dissolved in 0.02 mol dm^{-3} NaOD/D_2O (3 cm^3) and examined periodically by 270 MHz ^{1}H NMR spectroscopy. The 5-H proton of (3*S*,5*R*,6*S*)-benzylpenicillin at δ 5.24 appears as a singlet and there was no 6-H signal at δ 4.88 for this epimer. Epimerisation at C-6 in (3*S*,5*R*,6*R*)-benzylpenicillin therefore occurs with deuterium exchange at C-6. Coupling constants are in Hz.

(3S,5R,6R)-*Benzylpenicilloate* (**5**; R = $PhCH_2$). $\delta_H(D_2O)$ 1.21 (s, 3 H, 2-α-Me), 1.48 (s, 3 H, 2-β-Me), 3.41 (s, 1 H, 3-H), 3.67 (q, 2 H, $PhCH_2$), 4.23 (d, 1 H, 6-H, $J_{6,5}$ 6), 5.05 (d, 1 H, 3-H, $J_{5,6}$ 6) and 7.45 (m, 5 H, Ph).

(3S,5S,6R)-*Benzylpenicilloate* (**6**; R = $PhCH_2$). $\delta_H(D_2O)$ 1.02 (s, 3 H, 2-α-Me), 1.56 (s, 3 H, 2-β-Me), 3.39 (s, 1 H, 3-H), 3.78 (q, 2 H, $PhCH_2$), 4.76 (d, 1 H, 6-H, $J_{6,5}$ 3), 5.05 (d, 1 H, 5-H, $J_{5,6}$ 3) and 7.45 (m, 5 H, Ph).

(3S,5R,6R)-*Monomethyl benzylpenicilloate* (**8**). $\delta_H(D_2O)$ 1.22 (s, 3 H, 2-α-Me), 1.52 (s, 3 H, 2-β-Me), 3.59 (s, 1 H, 3-H), 3.79 (s, 2 H, $PhCH_2$), 3.84 (s, 3 H, OMe), 4.70 (d, 1 H, 6-H, $J_{6,5}$ 6), 5.20 (d, 1 H, 5-H, $J_{5,6}$ 6) and 7.37 (s, 5 H, Ph).

(3S,5S,6R)-*Monomethyl benzylpenicilloate.* $\delta_H(D_2O)$ 1.02 (s, 3 H, 2-α-Me), 1.58 (s, 3 H, 2-β-Me), 3.74 (s, 1 H, 3-H), 3.84 (s, 2 H, $PhCH_2$), 3.83 (s, 3 H, OMe), 5.20 (d, 1 H, 6-H, $J_{6,5}$ 3), 4.98 (d, 1 H, 5-H, $J_{5,6}$ 3) and 7.38 (s, 5 H, Ph).

(3S,5R,6R)-*Dimethyl benzylpenicilloate* (**9**). $\delta_H(CDCl_3)$ 1.12 (s, 3 H, 2-α-Me), 1.43 (s, 3 H, 2-β-Me), 3.32 (s, 1 H, 3-H), 3.62 (s, 2 H, $PhCH_2$), 3.71 (s, 6 H, 2 × OMe), 4.62 (dd, 1 H, 6-H, $J_{6,5}$ 4, J_{6NH} 9), 5.12 (d, 1 H, 5-H, $J_{5,6}$ 4), 6.20 (d, 1 H, NH) and 7.35 (s, 5 H, Ph).

(3S,5S,6R)-*Dimethyl benzylpenicilloate.* $\delta_H(CDCl_3)$ 0.85 (s, 3 H, 2-α-Me), 1.55 (s, 3 H, 2-β-Me), 3.49 (s, 1 H, 3-H), 3.64 (s, 2 H, $PhCH_2$), 3.70 (s 6 H, 2 × OMe), 5.05 (d, 1 H, 5-H, $J_{5,6}$ 2), 5.17 (dd, 1 H, 6-H, $J_{6,NH}$ 9), 6.30 (d, 1 H, NH) and 7.31 (s, 5 H, Ph).

HPLC.—All HPLC analyses were performed using a Kontron model 414 pump with a Kontron Uvikon 740 LC UV detector set at 254 nm. The derivatives could be separated using a reversed-phase system of non-polar Licrosorb octadecylsilyl RP C18 25 cm × 4 mm column and, as the eluent, aqueous acetonitrile containing 0.1% trifluoroacetic acid. Eluent and retention times (t_R/min) for each derivative were, respectively, in CH_3CN–H_2O (25:75) (5*R*,6*R*)-monomethyl benzylpenicilloate (**8**) 10.4, 5*S*,6*R* isomer 11.5; in CH_3CN–H_2O (35:65) (5*R*,6*R*)-dimethyl benzylpenicilloate (**9**) 10.7, 5*S*,6*R* isomer 11.9. Good resolution was also obtained using 5 μm polystyrene/divinylbenzene reverse-phase column and eluting with 80:20 (v/v) 0.02 mol dm^{-3} Na_2HPO_4, 0.004 mol dm^{-3} Na_3PO_4 in MeCN–H_2O to give retention times (t_R/min) for (5*R*,6*R*)-monomethyl benzylpenicilloate (**8**) and its 5*S*,6*R* epimer of 4.7 and 6.2, respectively. The dimethyl esters could be resolved eluting with MeCN–H_2O (40:60) with retention times (t_R/min) 12.4 and 14.2 for 5*R*,6*R* and 5*S*,6*R*, respectively.

Kinetics.—AnalaR grade chemicals were used exclusively in the preparation of buffers. Freshly boiled glass-distilled water was used throughout and the ionic strength maintained at 1.0 mol dm^{-3} with potassium chloride except where otherwise indicated. The eluent required for HPLC analysis was prepared from AnalaR grade chemicals and Hypersolv HPLC grade acetonitrile.

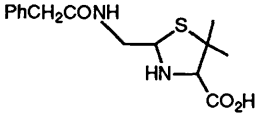
The pH of buffer solutions was measured with a Philips PW 9409 digital pH meter equipped with a Russel type CEL glass combination electrode calibrated against standard buffers of known pH at 30 °C.

The kinetic data were processed using a non-linear generalised least-squares program to calculate first-order rate constants using an iterative procedure and treating the rate constant and the initial and final values of substrate concentration as adjustable parameters. The rate constants for very slow reaction were determined from initial slopes.

Results and Discussion

Epimerically pure (3*S*,5*R*,6*R*)-benzylpenicilloate **5** was prepared from the hydrolysis of (3*S*,5*R*,6*R*)-benzylpenicillin using *B. cereus* β-lactamase as a catalyst. If the hydrolysis was carried out using dilute sodium hydroxide the product was always contaminated with traces of other epimers. Above pH 8 benzylpenicilloate is very stable in aqueous solution, there being no degradation products observable by HPLC after several days. The only observable reaction is epimerisation at C-5, followed by a much slower epimerisation at C-6.

Below pH 7 there is a slow degradation of benzylpenicilloic acid to benzylpenilloic acid **10** and, subsequently to D-penicillamine **11** and benzylpenilloaldehyde **12**.[15] However, the



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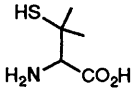

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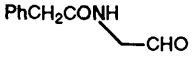

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rate of epimerisation at C-5 is at least 20 times greater than that of degradation, since less than 5% (HPLC and NMR) of these products were observed during the time course of epimerisation. (3*S*,5*R*,6*R*)-Benzylpenicilloate was prepared enzymatically because alkaline hydrolysis of (3*S*,5*R*,6*R*)-benzylpenicillin causes, in addition to hydrolysis, a small amount (<5%) of epimerisation at C-6 without ring opening. Epimerisation at C-6 in (3*S*,5*R*,6*R*)-benzylpenicillin to give the 3*S*,5*R*,6*S* epimer is accompanied by deuterium exchange at 6-H when the reaction is carried out in NaOD/D_2O. The 5-H proton of (3*S*,5*R*,6*S*)-benzylpenicillin at δ 5.24 appears as a singlet and there is no 6-H signal at δ 4.88 for this epimer. The second-order rate constant for this hydroxide-ion-catalysed reaction was not determined accurately but is, *ca.* 25 times smaller than that for hydrolysis *i.e.* it is *ca.* 6 × 10^{-3} dm^3 mol^{-1} s^{-1}. Epimerisation at C-6 in (3*S*,5*R*,6*R*)-benzylpenicilloate was negligible (<2%) under the conditions and time period used to study epimerisation at C-5.

The pH–rate profile for the epimerisation of (5*R*,6*R*)-benzylpenicilloate **5** to its 5*S*,6*R* epimer **6** is shown in Fig. 1. There are three distinct regions: at high pH a hydroxide ion catalysed reaction, between pH 6 and 12 a pH independent rate and at low pH an acid catalysed reaction which also becomes pH independent at low pH.

pH-*Independent Reaction.*—This reaction gives an equilibrium mixture of the two epimers and occurs with an observed first-order rate constant of 5.84 × 10^{-5} s^{-1}. From the methyl signals in the ^{1}H NMR spectrum and from HPLC data, the ratio of the concentration of the 5*S*,6*R* epimer to that of 5*R*,6*R* is 5.67 (85:15). If the reaction is carried out in D_2O, there is no incorporation of deuterium at either 5-H or 6-H of the products. There is little detectable epimerisation at C-6, and it

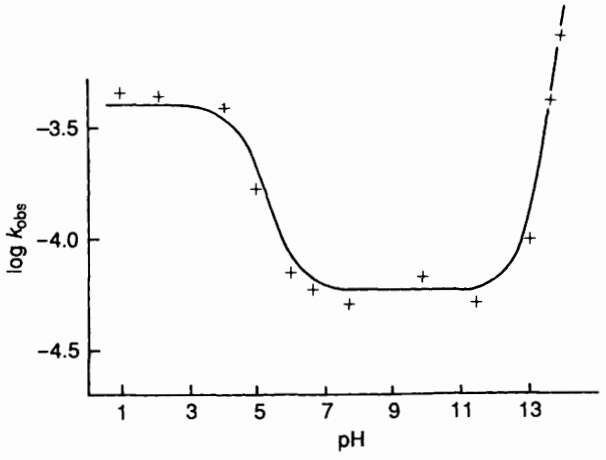
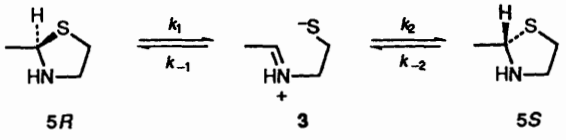


Fig. 1 The pH–rate profile for the epimerisation of (3*S*,5*R*,6*R*)-benzylpenicilloic acid in water at 30 °C. The line is calculated from eqn. (4) using the values given in the text and Table 1.

is estimated that there is less than 2% of the 6*S*,5*S* and 6*S*,5*R* epimers of benzylpenicilloate formed during the first 10 h. A continuous UV scan of the reaction mixture shows that there is no significant formation of the enamine **7**.

The pH-independent epimerisation at C-5 therefore probably occurs by unimolecular ring opening of the thiazolidine to give the iminium ion **3** (Scheme 1). Intramolecular thiolate ion



Scheme 1

attack on the iminium ion recloses the ring to give either the *R* or *S* epimer at C-5. Hydroxide ion adds to iminium ions with a rate constant of 10^6 dm^3 mol^{-1} s^{-1} [16] so *intramolecular* thiolate ion addition is expected to be very rapid. It may, of course, occur faster than the rate of conformational change and in this case the observed rate constant for epimerisation would give a lower limit to the rate of thiazolidine ring opening. Although ring closure is a 5-*endo-trig* process and is thought to be unfavourable[17] there are many examples where this occurs rapidly.[18,19] No hydrolysis products of the iminium ion are observed during epimerisation and therefore intramolecular thiolate addition occurs faster than attack by hydroxide ion. Similarly, ring closure to the thiazolidine occurs faster than intramolecular attack by the C-3 carboxylate to form an oxazolidinone.

The iminium ion **3** is probably not at equilibrium with respect to proton transfer. The pK_a of the thiol is estimated to be 7.9 whereas that of the iminium ion is 5.3.[20] Assuming that deprotonation/protonation is diffusion controlled in the thermodynamically favourable direction, protonation of the thiolate anion will occur by water above pH 6 and is estimated to have a rate constant of *ca.* 10^4 s^{-1}, whilst deprotonation of the iminium ion occurs by water below pH 8, with a rate constant of 10^4 s^{-1}, but above this pH occurs by hydroxide ion, with a second-order rate constant of *ca.* 10^{10} dm^3 mol^{-1} s^{-1}. As the epimerisation is still pH independent at pH 11 the rate of ring closure must be greater than 10^8 s^{-1}.

The observed first-order rate constant for the spontaneous epimerisation of (6*R*,5*R*)-benzylpenicilloate is the sum of the forward and back rates [eqn. (2)]. The forward rate constant is

$$6R,5R \underset{k_r^\circ}{\overset{k_f^\circ}{\rightleftharpoons}} 6R,5S \qquad (2)$$

then given by eqn. (3), so that the minimum rate constant for

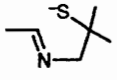
$$k_f^\circ = k_{obs}^\circ/(1 + 1/K^\circ) \qquad (3)$$

unimolecular cleavage of the C–S bond in the thiazolidine of benzylpenicilloate at 30 °C is 4.96 × 10^{-5} s^{-1}. The value of k_r° is 8.74 × 10^{-6} s^{-1} and the equilibrium constant K° = 5.67.

The rate of C-5 epimerisation of the mono- and di-methyl esters of (5*R*,6*R*)-benzylpenicilloate (**8** and **9**) also show a pH-independent reaction (Fig. 2). However, esterification of the carboxy groups reduces the rate of spontaneous thiazolidine ring opening. The observed pH-independent first-order rate constant for epimerisation of the dimethyl ester **9** is 3.53 × 10^{-8} s^{-1}, which is 1.7 × 10^3 times smaller than that observed for the dicarboxylate anion **5**. Esterification of the carboxylate groups makes them electron-withdrawing and it is therefore expected that thiazolidine ring opening, and the generation of positive charge at C-5 and N-4 in the iminium ion **3** (Scheme 1), should become unfavourable. This is also manifested in the reduced basicity of the thiazolidine nitrogen, the conjugate acid of which shows a pK_a 1.2 for the 5*R* epimer. A similar, but less marked, reduction in the rate of epimerisation and basicity of the thiazolidine nitrogen is observed for the monomethyl ester **8**. The rate of C-5 epimerisation of **8** is 21 times less than that of the dianion **5** whereas the pK_a of the *N*-conjugate acid of **8** is 3.80. Below pH 8 the rate of C-5 epimerisation of **8** is faster than hydrolysis of the methyl ester confirming our initial report[13] and contrary to a recent statement.[21] Below pH 8 the 5*R* and 5*S* epimers of **8** are clearly identifiable by NMR spectroscopy and HPLC. Continuous scanning of the NMR spectrum of **8** in aqueous (D_2O) solution at pD 7 shows a decrease in the C-2 methyl peaks of the 5*R* epimer at δ 1.22 and 1.52 and a growth of those for the 5*S* epimer at δ 1.02 and 1.58. The 6-H signal of the 5*R*,6*R* epimer at δ 4.70 decreases, whilst that of 5-H of the 5*S*,6*R* epimer at δ 4.98 increases.

Base-catalysed Reaction.—Base catalysed epimerisation of (5*R*,6*R*)-benzylpenicilloate **5** at C-5 occurs above pH 12.5 because the rate becomes dependent on hydroxide ion concentration (Fig. 1) with an observed second-order rate constant of 7.60 × 10^{-4} dm^3 mol^{-1} s^{-1}. There are several possible pathways to explain this dependence.

(*a*) The iminium ion intermediate **3** formed by uncatalysed cleavage of the thiazolidine could be deprotonated by hydroxide ion to give the thermodynamically more stable imine **13**. The rate of epimerisation then increases because the steady-state concentrations of the ring-opened thiazolidine increases.



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(*b*) The thiazolidine could undergo a concerted base-catalysed elimination process to form the neutral imine by hydroxide-ion abstraction of the hydrogen on the nitrogen **14**. This would avoid the formation of the unstable iminium ion.

(*c*) The benzylpenicilloate could undergo an elimination across C5–C6 to form an enamine **7**. If this was a concerted *anti* E2-type elimination ring closure would be expected to regenerate the 5*R*,6*R* epimer or give the 5*S*,6*S* configuration. The latter product is not observed. However, the appearance of a chromophore at 280 nm, consistent with the formation of the enamine **7** is observed at high base concentration (> 1 mol dm^{-3} NaOH). The absorption maximum and observed pseudo-first-order rate constants are dependent upon hydroxide-ion concentration and the calculated second-order rate constant for this process is 4.50 × 10^{-4} dm^3 mol^{-1} s^{-1}. Furthermore,

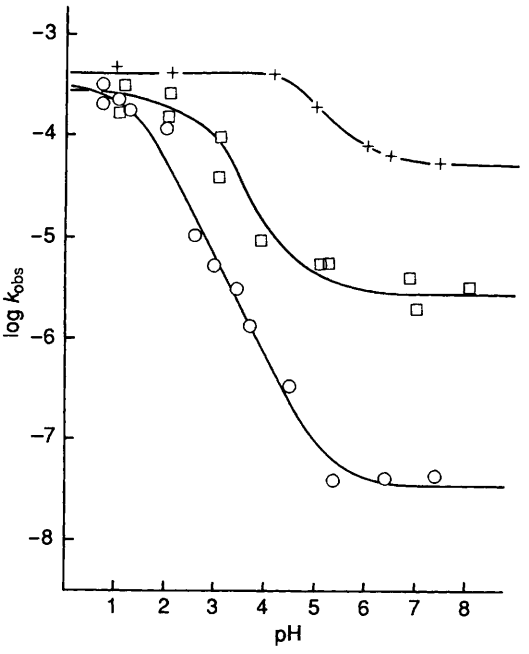


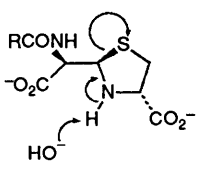
Fig. 2 The pH–rate profile for the epimerisation of (3*S*,5*R*,6*R*)-benzylpenicilloic acid (+), its monomethyl ester **8** (□) and its dimethyl ester **9** (○) in water at 30 °C. The lines are calculated from eqn. (4) using the values given in Table 1.

deuterium incorporation at 6-H of the penicilloate also occurs as indicated by the broadening of the 5-H doublet and the disappearance of 6-H in the NMR spectrum. For example, in 3.0 mol dm^{-3} NaOD/D_2O epimerisation at C-5 is *ca.* 60% complete after 4 min. After 14 min, the 6-H resonance of 5*R*,6*R* is no longer observable whilst those of 6-H and 5-H of 5*S*,6*R* are reduced in intensity and collapsing towards a singlet, respectively. After 26 min, these latter two processes are complete *i.e.* no resonance for 6-H and a singlet for 5-H.

It appears therefore, that two processes are being observed. Relatively fast epimerisation at C-5 *via* the imine followed by a slower epimerisation and deuterium exchange at C-6 *via* the enamine. The enamine **7** could either be formed directly from the intact thiazolidine **5** or from tautomerism of the iminium ion **3** or its conjugate base, **13**.

The base-catalysed epimerisation of benzylpenicilloate **5** is unlikely to occur *via* a unimolecular E1-type process as originally suggested.[13] At high pH it is conceivable that the rate of deprotonation of **3** by hydroxide ion is faster than the rate of ring closure and that therefore the steady-state concentration of the iminethiolate **13** increases. However, the rate-limiting step would still be unimolecular ring opening of the thiazolidine which would not account for the kinetic dependence of epimerisation upon base concentration.

The most likely mechanism for base-catalysed epimerisation of (5*R*,6*R*)-benzylpenicilloate is therefore a concerted E2-type mechanism (**14**) to form the neutral imine. The re-closure of the thiazolidine ring occurs by the microscopic reverse process involving general-acid catalysis by water (see **15**).



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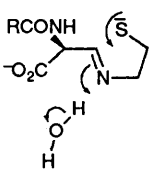

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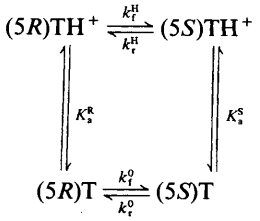
A similar process is the base-catalysed dehydration of carbinolamines to imines.[22] In this mechanism there is a large amount of proton transfer and charge development on the leaving oxygen atom.[23]

The base-catalysed reaction of the ester **8** is the subject of the following paper.[24]

Epimerisation at Low pH.—The observed pseudo first-order rate constant for epimerisation at C-5 increases at low pH and follows a sigmoidal curve (Fig. 1). The increase in rate passes through the pK_a of the protonated thiazolidine nitrogen **16**, TH^+. The 5*R* and 5*S* epimers have a different pK_a and the observed pseudo-first-order rate constant becomes a more complicated funcion of forward and reverse rates. The equilibria involved are outlined in Scheme 2, where (5*R*)T and (5*S*)T



Scheme 2

represent the 5*R*,6*R* epimers of the unprotonated thiazolidine of benzylpenicilloate, respectively, which interconvert with the rate constants k_f^0 and k_r^0.* The protonated thiazolidine epimers, **16**, (5*R*)TH^+ and (5*S*)TH^+, have dissociation constants K_a^R and K_a^S respectively, and interconvert with rate constants k_f^H and k_r^H. The equilibrium constant between the unprotonated thiazolidine epimers is K^0 and that between the protonated ones K^H, which equals $K_a^R K^0/K_a^S$. The observed pseudo-first-order rate constant k_{obs} is then given by eqn. (4) where α^R and α^S represent

$$k_{obs} = k_f^H(1 - \alpha^R) + \frac{k_f^H}{K^H}(1 - \alpha^S) + k_f^0\alpha^R + \frac{k_f^0\alpha^S}{K^0} \quad (4)$$

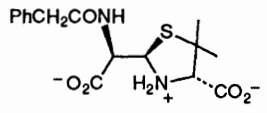
the fractions of unprotonated 5*R* and 5*S* epimers. The line in Fig. 1 is generated using the parameters given in Table 1. The change in equilibrium constant between the 5*R*,6*R* and 5*S*,6*R* epimers for the unprotonated and protonated thiazolidines from 5.67 to 1.60, respectively, corresponds to a change in ratio of 85% 5*S*,6*R*:15% 5*R*,6*R* to 60% 5*S*,6*R*:40% 5*R*,6*R*, respectively. There is a smaller difference in the energies of the two epimers when the thiazolidine nitrogen is protonated.

The increase in the rate of epimerisation at low pH is interesting. It is difficult to believe that the mechanism involves the unimolecular ring opening of the protonated thiazolidine **16**. Epimerisation could occur *via* either the kinetically equivalent form of the unprotonated thiazolidinecarboxylic acid **17** or the *S*-protonated thiazolidine **18**. If **17** is the active form of the substrate then the calculated rate constant for epimerisation would be *ca.* 2×10^{-2} s^{-1} *i.e. ca.* 400 times greater than that for the spontaneous, or water-catalysed, reaction. This rate enhancement could be attributable to intramolecular general-acid catalysis **19** or **20**. However both the monomethyl ester of (3*S*,5*R*,6*R*)-benzylpenicilloate (**8**) and the dimethyl ester **9** show a similar pH-dependent rate of epimerisation (Fig. 2). It is therefore unlikely that the higher rate of epimerisation of benzylpenicilloate at low pH is due to intramolecular general-acid catalysis. In fact the pH independent rate at low pH is similar for all three derivatives. This presumably arises from the similar electronic effects of the ester and undissociated carboxy

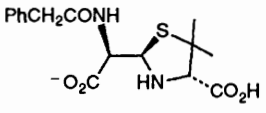
* This simplified scheme ignores the effect of the ionisation of the carboxyl groups which will become important at very low pH.

Table 1 Rate and equilibrium constants for the C-5 epimerisation of (3*S*,5*R*,6*R*)-benzylpenicilloic acid (**5**), its methyl ester (**8**) and dimethyl ester (**9**) in water at 30 °C

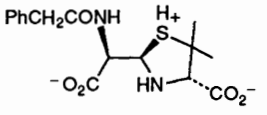
Derivative	k^{0}_{obs}/s^{-1}	k^{0}_{f}/s^{-1}	k^{0}_{r}/s^{-1}	K^{0}	k^{H}_{obs}/s^{-1}	k^{H}_{f}/s^{-1}	k^{H}_{r}/s^{-1}	K^{H}	pK^{R}_{a}	pK^{S}_{a}
Diacid **5**	5.84×10^{-5}	4.96×10^{-5}	8.74×10^{-6}	5.67	3.98×10^{-4}	2.45×10^{-4}	1.53×10^{-4}	1.60	5.14	4.59
Monoester **8**	2.82×10^{-6}	1.12×10^{-6}	1.70×10^{-6}	0.66	2.37×10^{-4}	1.42×10^{-4}	9.48×10^{-5}	1.50	3.80	3.35
Diester **9**	3.53×10^{-8}	1.39×10^{-8}	2.14×10^{-8}	0.65	3.16×10^{-4}	1.84×10^{-4}	1.31×10^{-4}	1.40	1.18	1.51



16



17



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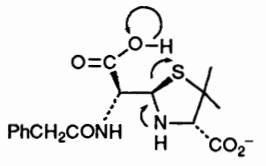

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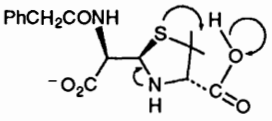
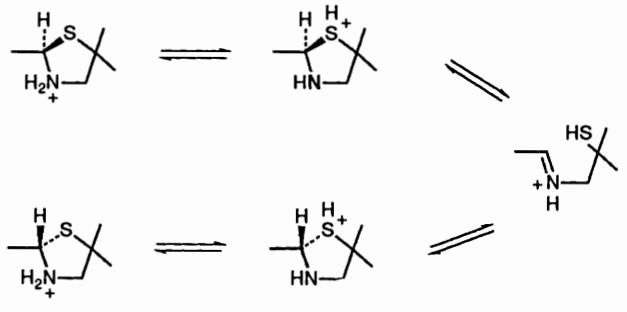
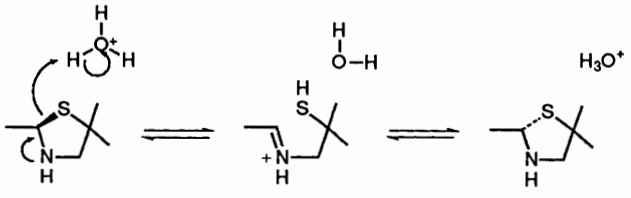


20

groups. The rate of C-5 epimerisation of the dimethyl ester **9** increases markedly at low pH (Fig. 2) so that the pH independent rate at pH 1 is 10^4 times greater than that at pH 7 (Table 1). The calculated rate and equilibrium constants using Scheme 2 for the two esters are given in Table 1. As expected, the pK_a of the protonated thiazolidine is decreased by esterification so the rate does not level off for the diester until very low pH (< 1) is reached. The mechanism of the acid-catalysed reaction is presumably as outlined in Scheme 3 or the kinetically equivalent concerted process in Scheme 4.



Scheme 3



Scheme 4

Acknowledgements

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