Aqueous and Anhydrous Degradations of 6α-Formamidopenicillins

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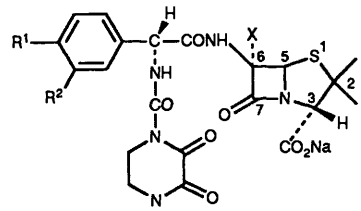
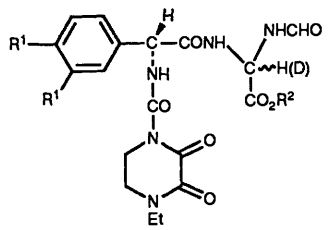
When an aqueous solution of the 6α-formamidopenicillin BRL 36650 (**1**) was set aside for 36 h, an essentially quantitative C(5)–C(6) cleavage resulted, yielding the α,α-bis(acylamino) acid (**6**) and *N*-formylpenicillamine (**10**). The unsubstituted phenyl compound (**2**) behaved analogously, but the 4-aminophenyl derivative (**3**) showed five-fold greater aqueous stability. Over a wider range of pH, the penicillin (**1**) was converted into the penillic acid (**21**) and/or the penicilloic acid (**22**) at $1 < pH < 7$ and into the piperazine ring-opened diacid (**23**) at pH 10.

Both the 6β-aminopenicillin ester (**15**) and the urethane (**24**) yielded penicilloates (**25**) and (**26**) on base-catalysed methanolysis. However, while (**15**) was recovered in good yield after prolonged treatment with triethylamine, (**24**) underwent C(5)–C(6) cleavage, forming the dihydrothiazole (**27**). These results are interpreted in terms of initial oxazolone formation *via* the 6β-amido substituent, followed by C(5)–C(6) bond breaking.

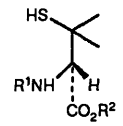
We have previously disclosed 6α-formamidopenicillins such as BRL 36650 (**1**),[1] outlined their synthesis,[2] and demonstrated their excellent activity against Gram-negative bacteria.[3,4] The tendency of 6α-formamidopenicillins to undergo degradation *via* C(5)–C(6) cleavage under mild conditions has also been reported.[5] We now present a fuller account of the aqueous degradations of 6α-formamidopenicillins like (**1**) of the acylureido type and the anhydrous degradation of some simpler 6α-formamidopenicillins together with some mechanistic interpretation of our results.



(**1**) $R^1 = R^2 = OH$, X = NHCHO
(**2**) $R^1 = R^2 = H$, X = NHCHO
(**3**) $R^1 = NH_2$, $R^2 = H$, X = NHCHO
(**4**) $R^1 = R^2 = X = H$



(**5**) $R^1 = OH$, $R^2 = CHPh_2$
(**6**) $R^1 = OH$, $R^2 = H$ or Na
(**7**) $R^1 = H$, $R^2 = CHPh_2$



(**8**) $R^1 = OHC$, $R^2 = CHPh_2$
(**9**) $R^1 = R^2 = H$
(**10**) $R^1 = OHC$, $R^2 = H$
(**11**) $R^1 = OHC$, $R^2 = CH_2Ph$

Aqueous Degradations.—When a 3% aqueous solution of the penicillin (**1**), initial pH 6.2, was left at ambient temperature, UV-monitored TLC or HPLC analysis showed complete loss of (**1**) after 36 h with conversion to very largely a single, more polar, UV-absorbing product. The final pH was 2.5. Extraction of acidic material, then esterification with diphenyldiazomethane, afforded by precipitation then chromatography the esters (**5**), as an inseparable 1:1 diastereoisomeric mixture, and the penicillamine derivative (**8**). The former were readily characterised by spectroscopic and analytical data; acidolysis regenerated the epimeric acids (**6**), also fully characterised. The latter proved identical (m.p.; rotation) to material obtained by formylation[6] and esterification of D-penicillamine (**9**). Inefficient extraction of the free acids (**6**) and (**10**) into organic solvents led to only modest yields of the esters (**5**) and (**8**). However, NMR analysis readily showed that the conversion (**1**)→(**6**) + (**10**) was in fact virtually quantitative. Thus a D_2O solution of (**1**) at the same concentration gave the spectra shown in Figure 1 over a 36 h period. This series also illustrates a pronounced rate acceleration, from about 20% loss of (**1**) after 12 h to about 80% after 20 h. Together with the pH drop during the process (Figure 2), this implies a process autocatalytic in acid. Note that in the NMR experiments the acids (**6**) bear a deuterium atom not seen in the spectra. Our mechanistic interpretation of these data is given in Scheme 1; one important function of the 6α-formamido group is to block penicillenic acid formation[7a,b] and direct the postulated initial oxazolone (**28**) to other pathways. The production of *N*-formylpenicillamine (**10**) from aqueous degradation of some 6α-H penicillins is known;[8,9] it is not so uncommon as once thought, but from 6α-H penicillins of the (acylureido) class such as piperacillin (**4**) it occurs to less than 0.1% under comparable conditions.[9]

We examined the behaviour of the unsubstituted phenyl penicillin (**2**)[1,3] under comparable conditions. It underwent an analogous reaction at a similar rate, giving after work-up the ester (**8**) and the C(2)-epimeric esters (**7**) which in this series were chromatographically separable. In other words, the catechol group of (**1**) was inessential for the degradation despite its mildly acidic character. We speculated, however, that the amino analogue (**3**) might exhibit greater aqueous stability through internal buffering by the amino group. Synthesis of (**3**) began with a high-yielding two-phase acylation of aniline giving the urethane (**12**). Amidoalkylation of (**12**) with the known[10] α-hydroxyglycine derivative (**13**) was achieved using the conditions of Ben-Ishai and co-workers.[11] The desired acid (**14**) was obtained together with its *ortho*-isomer in a 4:3 ratio and 40% total yield; (**14**) was isolated by crystallization. *N,N'*-Dicyclo-

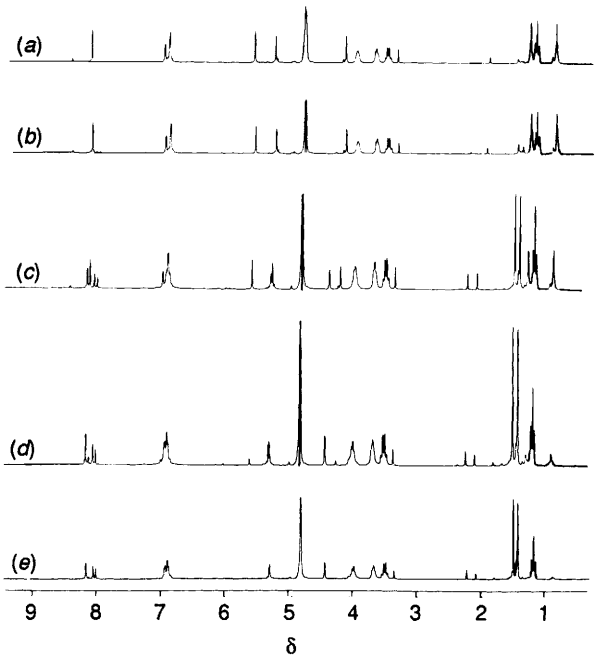


Figure 1. NMR spectrum of a 3% solution of (**1**) in D_2O as a function of time. The peak at δ 3.35 is caused by a trace of methanol; (*a*) initial; (*b*) after 12 h; (*c*) 24 h; (*d*) 28 h; (*e*) 36 h.

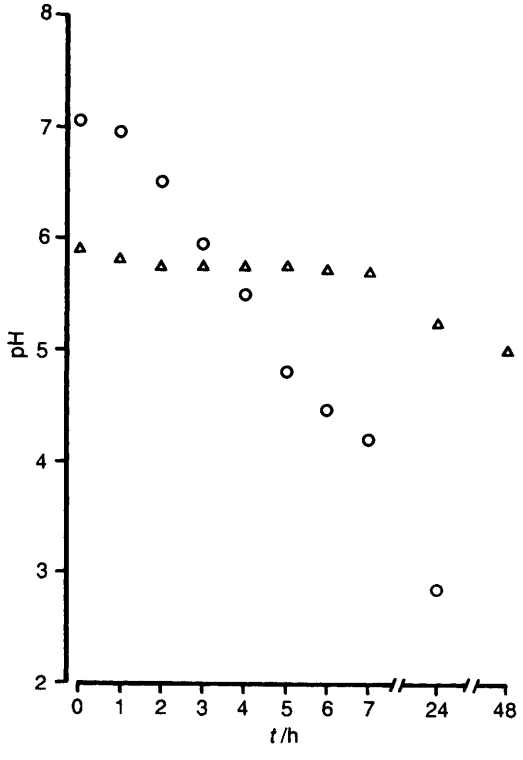
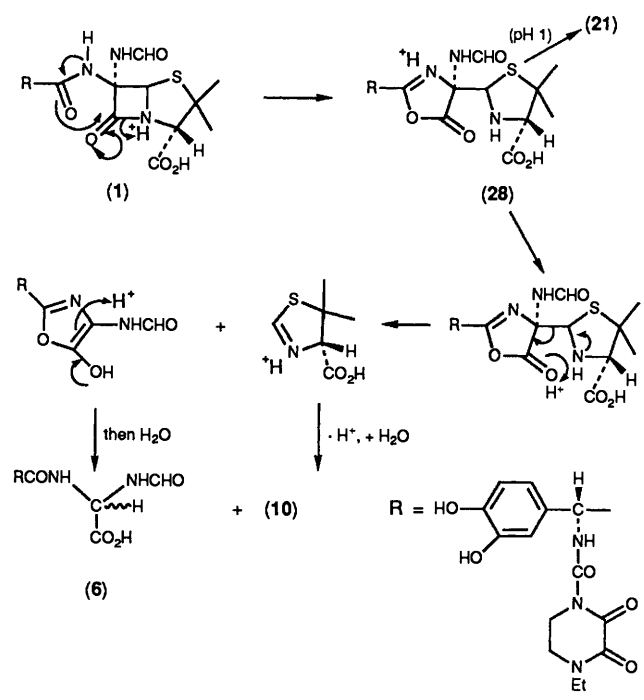


Figure 2. The pH variation of 10% aqueous solutions of penicillins (**1**) (○) and (**3**) (△) with time.

hexylcarbodi-imide mediated coupling of acid (**14**) to the 6α-formamidopenicillin nucleus (**15**)[1,2] gave an approximately 1:1 (*R,S*) mixture of the esters (**17**) in 63% yield. Chromatographic separation of the (*R*)-epimer,* then hydrogenolysis, afforded the penicillin (**3**) [29% from (**14**)]. HPLC and NMR analysis of a 10% aqueous solution of (**3**) showed it to be appreciably more stable than (**1**), being less than 20%



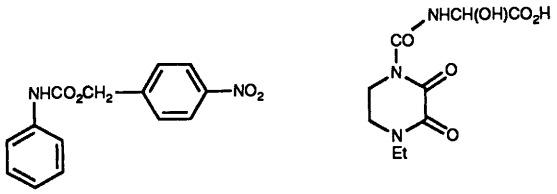
Scheme 1. Aqueous degradation of (**1**) at 'natural' pH.

degraded after 48 h; moreover, the pH of aqueous (**3**) remained significantly higher, Figure 2. When the degradation of (**3**) was complete (*ca.* 7 days), a complex mixture of products resulted (HPLC; NMR).

Returning to the penicillin (**1**), we also examined its aqueous degradation over a range of pH values. Dissolution of (**1**) in 1M hydrochloric acid gave a rapid, high-yielding conversion to the penillic acid (**21**). However, for ease of isolation of this rather labile substance in a pure form, it was necessary to proceed *via* protected intermediates. Thus the acid (**18**)[1] was protected as its bis(benzyl carbonate) (**19**) and coupled to the 6α-(methylthio)-penicillin nucleus (**16**).[12] Treatment of the crude product with *N,N*-bis(trimethylsilyl)formamide[2,13] afforded the ester (**20**) (43%) which on acidolysis with 1M hydrochloric acid followed by hydrogenolysis gave the penillic acid (**21**) (71%). Since the NMR spectrum of (**21**) showed an intact formamido group the alternative penillic acid structure (**21'**) could be ruled out. Taking into account the accepted mechanism of penillic acid formation,[14] this again implies that the 6β-acylamino, not the 6α-formamido group, is involved in the initial oxazolone formation (Scheme 1, arrows). Aqueous solutions of the penillic acid (**21**), even its freeze-dried form, were distinctly unstable; neutralization of aqueous (**21**) to pH 7 gave a clean conversion to the penicilloic acid (**22**) [characteristic signal at $\delta(D_2O)$ 3.11 (1 H, s)], but reacidification to pH 1 reversed this process. Aqueous solutions of the penicillin (**1**) at $1 < pH < 7$ were degraded to mixtures of (**6**), (**10**), (**21**), and (**22**). When aqueous (**1**) was briefly set aside at pH 10, the 2,3-dioxopiperazine ring opened yielding the oxamic acid penicillin (**23**) in keeping with the behaviour of piperacillin (**4**).[15] These pathways are summarised in Scheme 2.

Anhydrous Degradations.—We also examined the chemistry of some simpler 6α-formamidopenicillins under anhydrous conditions. Both the aminopenicillin ester (**15**) and its

* As with all 6α-formamidopenicillin esters we have studied, the (*R*)-epimer of (**17**) was more polar and showed greater separation of the 2-methyl groups by ^1H NMR spectroscopy (see Experimental section).



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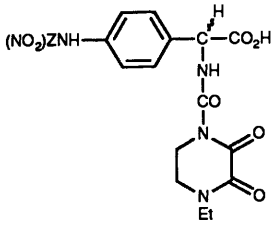

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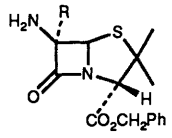
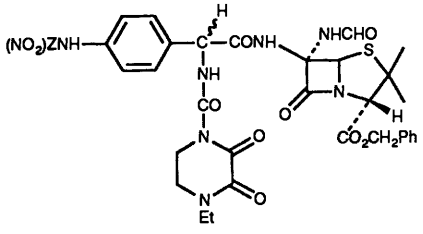
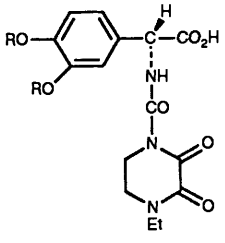


(15) R = NHCHO
(16) R = SMe

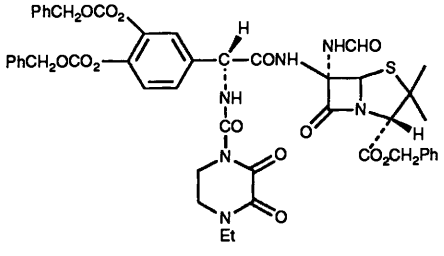


(17)

$(NO_2)Z$ = *p*-$O_2NC_6H_4CH_2OCO$.



(18) R = H
(19) R = $PhCH_2OCO$



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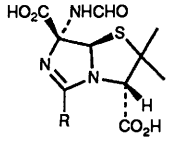

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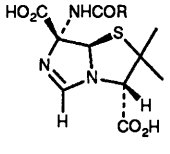

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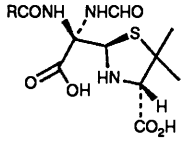
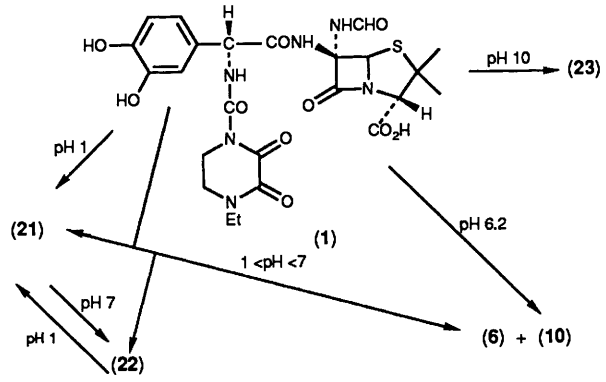


(22)

R as in Scheme 1.

(2,2,2-trichloroethoxy)carbonyl protected form (**24**)[1,2] showed interesting behaviour which shed further light on the above results. Reaction of (**15**) and (**24**) with triethylamine and methanol at 20 °C for 16 h afforded the respective methyl



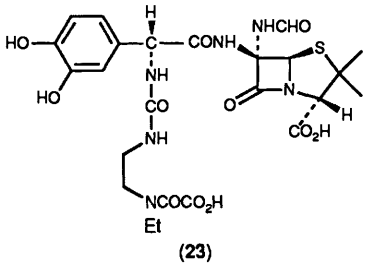
Scheme 2. Aqueous degradation products of (**1**).

penicilloates (**25**) and (**26**) both in about 70% yield. On treatment with triethylamine in an inert solvent, however, (**15**) gave largely unchanged starting material even after 7 days while (**24**) gave the dihydrothiazole (**27**) after 16 h. The latter compound was isolable on rapid chromatography, but traces of water caused efficient hydrolysis to the *N*-formylpenicillamine ester (**11**). Previously thiazolines have been detected from anhydrous acid treatment of penicillin G esters.[16] A mechanism similar to that in Scheme 1 may be postulated for the formation of the thiazole (**27**), with base rather than acid catalysis; formation of oxazolones from carboxy-activated α-urethano acids is well precedented.[17,18] These results support the contention that a 6β-acylamino or -urethano substituent is necessary for C(5)–C(6) cleavage; the 6α-formamido group appears unfavourably oriented for oxazolone formation.

Experimental

Organic extracts were finally washed with saturated brine and dried over anhydrous magnesium sulphate prior to rotary evaporation at or below 30 °C under reduced pressure. M.p.s were determined in a Büchi oil-immersion apparatus and are uncorrected. Optical rotations were measured in a 1 dm cell using a Perkin-Elmer 141 polarimeter at the sodium D-line. Unless otherwise noted, IR spectra were recorded for KBr discs in a Perkin-Elmer 457 instrument. ^{1}H NMR spectra were recorded on a Bruker WM250 instrument at 250 MHz unless otherwise stated, using an appropriate internal standard in the solvent quoted; all δ values quoted are δ_H. Formamido compounds showed rotational isomerism by ^{1}H NMR spectroscopy; for simplicity, only the major *Z*-rotamer is quoted (typically *Z*:*E* *ca.* 5:1). Mass spectra were recorded using a VG7070 instrument for the electron-impact mode (EI) or a VGZAB instrument for the fast-atom bombardment mode (FAB). Homogeneity of products was assessed by TLC on Merck silica gel 60 F_{254} plates, and by analytical HPLC on a Waters μ Bondapak™ C_{18} reverse-phase column where appropriate. Preparative chromatography was performed on Merck silica gel 7729 (finer than 230 mesh ASTM). Certain non-crystalline compounds for which accurate mass measurement by EIMS was not possible were regarded as adequately characterised by homogeneity, spectral data, and FAB MS measurement of the molecular ions.

(2S)-2-*Formamido*-3-*mercapto*-3-*methylbutanoic Acid* (N-*Formyl*-D-*penicillamine*) (**10**).—(2*S*)-2-Amino-3-methyl-3-mercaptobutanoic acid (**9**) (D-penicillamine; 2.24 g, 15.0 mmol) was *N*-formylated according to the procedure of Ghuysen *et al.*[6] Recrystallisation from hot water afforded the title acid (**10**) (1.34 g, 51%) in two crops; crop 1, m.p. 144–147 °C; $[\alpha]_D^{20}$



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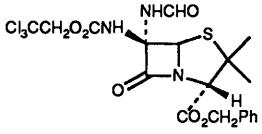

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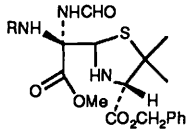
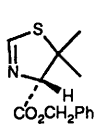


(25) R = H
(26) R = $Cl_3CCH_2O_2C$



(27)

+60.3° (*c* 1 in pyridine), +24.9° (*c* 2 in 1M phosphate buffer, pH 7.0) {lit.,[6] m.p. 146–148 °C; $[\alpha]_D^{20}$ +63.0° (*c* 1 in pyridine), +23.0° (*c* 2 in 1M phosphate buffer, pH 7.0}. The crystals from the second crop had significantly lower rotation and were not used further.

Diphenylmethyl (2S)-2-*Formamido*-3-*methyl*-3-*mercaptobutanoate* (**8**).—A solution of the above acid (**10**) (0.355 g, 2 mmol) in tetrahydrofuran (THF) (10 ml) was treated with 0.2M diphenyldiazomethane in CH_2Cl_2 (16 ml) and stirred at ambient temperature for 65 h, when no starting acid was visible by TLC. After destroying excess of reagent using glacial acetic acid (2 drops), the mixture was concentrated to low volume, diluted with ethyl acetate, and washed with saturated aqueous sodium hydrogen carbonate, then with water. Evaporation followed by chromatography of the residue, with 2.5% methanol in chloroform as eluant, gave the title *ester* (**8**) (0.305 g, 45%), m.p. 93—94 °C (from ethyl acetate–hexane) (Found: C, 66.4; H, 6.1; N, 4.1; S, 9.4. $C_{19}H_{21}NO_3S$ requires C, 66.5; H, 6.1; N, 4.1; S, 9.3%); $[\alpha]_D^{20}$ +42.1° (*c* 1 in chloroform); ν_{max} 3 220br, 1 740, 1 665, 1 535, and 1 495 cm^{-1}; $\delta(CDCl_3)$ 1.34, 1.52 (6 H, 2 × s, Me_2C), 1.86 (1 H, s, SH; a very small long-range SH to CH_3 coupling, *J ca.* 1 Hz, was observed), 4.87 (1 H, d, *J* 9 Hz, NHC*H*CO), 6.55 (1 H, br d, *J* 9 Hz, N*H*CHCO), 6.93 (1 H, s, Ph_2CHO), 7.25–7.40 (10 H, br s, ArH), and 8.28 (1 H, br s, NHC*H*O).

Diphenylmethyl (2RS)-2-{(2R)-2-(3,4-*Dihydroxyphenyl*)-2-[(4-*ethyl*-2,3-*dioxopiperazin*-1-*yl*)*carbonylamino*]*acetamido*}-2-*formamidoacetate* (**5**).—Sodium 6α-formamido-6β-{(2*R*)-2-(3,4-dihydroxyphenyl)-2-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]acetamido}penicillanate[1] (**1**) (1.0 g, 1.63 mmol) dissolved in water (30 ml) was kept at ambient temperature for 60 h. During this time the solution pH fell from 6.2 to 2.5; no penicillin was finally detected by HPLC. The solution was acidified to pH 2.0 using 2M hydrochloric acid, saturated with sodium chloride, and extracted with ethyl acetate–THF (1:1; 4 × 50 ml). After evaporation, the residue was dissolved as far as possible in THF (30 ml) and dimethylformamide (DMF) (10 ml) and treated with 1M diphenyldiazomethane in THF (1.8 ml). After 16 h at ambient temperature, work-up as for (**8**) gave a crude product (0.8 g) which on trituration with ether deposited a solid (0.162 g), the mother liquors being retained. Chromatography, with methanol–chloroform mixtures as eluant, followed by reprecipitation from THF–n-hexane gave the hygroscopic *epimeric esters* (**5**) (0.120 g) as a virtually 1:1 (2*RS*)-mixture, m.p. 150–155 °C (Found: C, 59.2; H, 5.4; N, 11.3. $C_{13}H_{31}N_5O_9 \cdot 0.5H_2O$ requires C, 59.4; H, 5.1; N, 11.2%); ν_{max} 1 745sh, 1 710, 1 675, and 1 610m cm^{-1}; $\delta[(CD_3)_2CO]$ 1.17 (3 H, t, *J* 7 Hz, CH_3CH_2N), 3.50 (2 H, q, *J* 7 Hz, CH_3CH_2N), 3.67, 4.02 (4 H, 2 × m, 2 × CH_2N), 5.46 (1 H, d, C*H*NH), 6.00–6.15 (1 H, m, dd on brief D_2O exch., NHC*H*NHCHO), 6.70–6.90 (3 H, m, ArH), 6.98 (1 H, s, Ph_2CHO), 7.10–7.50 (15 H, m, ArH), 8.17 (1 H, br s on D_2O exch., NHC*H*O), 8.35, 8.68, and 9.85 (3 H, 3 × m, D_2O exch., 3 × NH). Evaporation of the earlier mother liquors followed by chromatography, with methanol–chloroform as eluant, afforded the *N*-formyl penicillamine ester (**8**) (0.158 g) which on crystallisation (ethyl acetate–hexane) had identical m.p., rotation, and spectral data to those of the above-mentioned synthetic material.

Sodium (2RS)-2-{(2R)-2-(3,4-*Dihydroxyphenyl*)-2-[(4-*ethyl*-2,3-*dioxopiperazin*-1-*yl*)*carbonylamino*]*acetamido*}-2-*formamidoacetate* (**6**).—The epimeric esters (**5**) (0.200 g, 0.32 mmol) were dissolved in trifluoroacetic acid (3 ml) and, after 1 h at ambient temperature, evaporated to dryness, then co-evaporated from chloroform (5 × 10 ml). Trituration with ether afforded on filtration a near-colourless solid (0.122 g) which was treated with a solution of sodium hydrogen carbonate (0.023 g) in water (10 ml). The aqueous solution was washed with ether, then lyophilized to give the very hygroscopic *epimeric sodium salts* (**6**) (0.135 g) (Found: C, 42.5; H, 4.6; N, 13.5. $C_{18}H_{20}N_5NaO_9 \cdot 2H_2O$ requires C, 42.4; H, 4.7; N, 13.75%); ν_{max} 1 710, 1 674, 1 630sh, and 1 506 cm^{-1}; $\delta(D_2O)$ 1.16 (3 H, t, *J* 7 Hz, CH_3CH_2N), 3.47 (2 H, q, *J* 7 Hz, CH_3CH_2N), 3.63, 3.95 (4 H, 2 × m, 2 × CH_2N), 5.25, 5.26 (1 H, 2 × s, ArC*H*NH), 5.48, 5.64 (1 H, 2 × s, NHC*H*NH), 6.75–6.95 (3 H, m, ArH), 7.98, and 8.03 (1 H, 2 × s, NHC*H*O); *m/z* (positive FAB; Carbowax) MH^+ (free acid), 452.

NMR Study of the Degradation of (**1**).—The NMR spectrum of a 3% solution of the penicillin (**1**) in D_2O was recorded at intervals over 36 h as shown in Figure 1. The conversion (**1**)→(**6**) + (**10**) was demonstrated, in particular, by the disappearance of the C(2) methyl signals (δ 0.9, 1.2) of (**1**) and the appearance of two new methyl singlets (δ 1.3, 1.4); also by the disappearance of the formamido H signal (δ 8.1) with the appearance of new signals at δ 8.0, 8.05, and 8.2.

Diphenylmethyl (2RS)-2-{(2R)-2-[(4-*Ethyl*-2,3-*dioxopiperazin*-1-*yl*)*carbonylamino*]-2-*phenylacetamido*}-2-*formamidoacetate* (**7**).—Sodium 6α-formamido-6β-{(2*R*)-2-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]-2-phenylacetamido}-penicillanate[1] (**2**) (0.400 g, 0.69 mmol) in water (12 ml) was left at ambient temperature for 72 h, when no starting material was visible by TLC. Work-up and esterification as for (**1**) gave a crude product (0.730 g) which was triturated with ether, giving a white precipitate. The solid was filtered off (the mother liquors being retained) then reprecipitated from chloroform–ether to give the title *esters* (**7**) as a 1:1 (2*RS*) mixture (0.199 g), m.p. 130–134 °C (Found: C, 62.8; H, 5.3; N, 11.0. $C_{31}H_{31}N_5O_7 \cdot 0.5H_2O$ requires C, 62.6; H, 5.4; N, 11.8%); ν_{max} 1 750, 1 715, 1 680, and 1 500 cm^{-1}; $\delta[(CD_3)_2CO]$ 1.14 (3 H, t, CH_3CH_2N), 3.46 (2 H, q, CH_3CH_2N), 3.64, 4.00 (4 H, 2 × m, 2 × CH_2N), 5.63 (1 H, 2 × d, C*H*NH), 5.95–6.05 (1 H, m, 2 × d on brief D_2O exch., NHC*H*NHCHO), 6.75 (1 H, s, Ph_2CHO), 7.10–7.50 (15 H, m, ArH), 8.13 (1 H, br s, NHC*H*O), 8.35, 8.80, and 9.95 (3 H, 3 × m, 3 × NH). By chromatography, with 5% methanol–chloroform as eluant, a single isomer of (**7**) was isolated, m.p. 148–154 °C (from ethyl acetate–hexane) (Found: C, 63.2; H, 5.5; N, 11.7. $C_{31}H_{31}N_5O_7$ requires C, 63.6; H, 5.3; N, 12.0%). Chromatography of the earlier mother liquors, with 2.5% methanol–chloroform as eluant, yielded the *N*-formylpenicillamine ester (**8**) (0.099 g) which on crystallisation from ethyl acetate–hexane had identical m.p. and rotation to those of the above synthetic material.

4-Nitrobenzyl Phenylcarbamate (**12**).—To a vigorously stirred two-phase system of aniline (2.33 g, 25 mmol) in ethyl acetate (50 ml) plus saturated aqueous sodium hydrogen carbonate (35 ml) and water (15 ml) at 0 °C was added in portions 4-nitrobenzyl chloroformate (5.39 g, 25 mmol). After 0.25 h complete dissolution had occurred; the organic phase was separated, washed further with dil. hydrochloric acid (× 2), dil. aqueous sodium hydrogen carbonate (× 2), and water, and evaporated to give the practically pure *urethane* (**12**) (6.72 g, 98%), m.p. 124–125 °C (from ethyl acetate–hexane) (Found: C, 61.4; H, 4.7; N, 10.4. $C_{14}H_{12}N_2O_4$ requires C, 61.75; H, 4.4; N, 10.3%); ν_{max}(Nujol) 3 350, 1 705, 1 600, 1 530, 1515, and 1 350 cm^{-1}; δ[60 MHz; $(CD_3)_2SO$] 5.25 (2 H, s, $ArCH_2O$), 6.80–7.40 (5 H, m, ArH), 7.50, 8.15 (4 H, dd, ArH), and 8.60 (1 H, br s, D_2O exch., NH).

(2RS)-2-[(4-*Ethyl*-2,3-*dioxopiperazin*-1-*yl*)*carbonylamino*]-2-{4-[(4-*nitrobenzyl*)*oxycarbonylamino*]*phenyl*}*acetic Acid* (**14**).—The urethane (**12**) (1.36 g, 5 mmol) and (2*RS*)-2-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]-2-hydroxyacetic acid (**13**)[10] (1.30 g, 5 mmol) were stirred together in a mixture of glacial acetic acid (5 ml) and conc. sulphuric acid (5 ml) for 1 h. The solution was poured onto ice–water and extracted with ethyl acetate (× 2). Following evaporation to dryness, the crude product was chromatographed on silica gel, with ethyl acetate–propan-2-ol–water mixtures (15:3:1, then 10:3:1, 5:3:1) as eluant. Appropriate fractions were pooled and evaporated to give a gum which on trituration with ethyl acetate slowly solidified; the white solid was filtered off, washed with ethyl acetate, and dried to give the *title acid* (**14**) (0.61 g, 24%), m.p. 129–134 °C (from dil. aqueous sodium hydrogen carbonate–dil.hydrochloric acid) (Found: C, 52.7; H, 5.4; N, 13.1%; MH^+, 514.1583. $C_{23}H_{23}N_5O_9 \cdot 0.5H_2O$ requires C, 52.8; H, 4.6; N, 13.4%; MH^+, 514.1574); ν_{max} 1 711, 1 678, 1 606, 1 520, and 1 347 cm^{-1}; δ[$(CD_3)_2SO$] 1.08 (3 H, t, *J* 7 Hz, CH_3CH_2N), 3.40 (2 H, q, *J* 7 Hz, CH_3CH_2N), 3.55, 3.90 (4 H, 2 × m, 2 × CH_2N), 5.27 (1 H, d, *J* 6.5 Hz, CHNH), 5.31 (2 H, s, $ArCH_2O$), 7.31, 7.48 (4 H, dd, ArH), 7.69, 8.28 (4 H, dd, ArH), 9.77 (1 H, d, *J* 6.5 Hz, CHN*H*), and 10.01 (1 H, br s, ArN*H*). Evaporation of the mother liquors gave a non-crystalline solid (0.47 g, 18%) which NMR spectroscopy showed was very largely the corresponding *ortho*-isomer: δ[$(CD_3)_2SO$], *inter alia*, 5.69 (1 H, d, *J* 6.5 Hz, CHNH), 7.20–7.60 (4 H, m, ArH), and 9.80 (1 H, d, *J* 6.5 Hz, CHN*H*).

Benzyl 6β-[(2R)-2-[(4-*Ethyl*-2,3-*dioxopiperazin*-1-*yl*)*carbonylamino*]-2-{4-[(4-*nitrobenzyl*)*oxycarbonylamino*]*phenyl*}-*acetamido*]-6α-*formamidopenicillanate* (**17**).—Benzyl 6β-amino-6α-formamidopenicillanate (**15**) (0.279 g, 0.80 mmol)[1] and *N*,*N*′-dicyclohexylcarbodi-imide (0.164 g, 0.80 mmol) were stirred together at 0 °C in ethyl acetate (2.5 ml). A solution of the protected acid (**14**) (0.420 g, 0.80 mmol) in ethyl acetate (2 ml) and DMF (2 ml) was added dropwise over 0.25 h, then the mixture was allowed to regain ambient temperature and stirring continued for 3 h. The precipitate was filtered off and washed well with ethyl acetate, then the filtrate was washed successively with dil. hydrochloric acid, dil. aqueous sodium hydrogen carbonate, and water. Evaporation followed by chromatography on silica gel, with methanol–chloroform mixtures as eluant, pooling, and evaporation of appropriate fractions afforded the (2R)-penicillin ester (**17**) (0.219 g, 32%); ν_{max} 1 785, 1 740sh, 1 710, 1 683, 1 606m, 1 520, and 1 347 cm^{-1}; δ[$(CD_3)_2CO$] 0.97, 1.17 (6 H, 2 × s, Me_2C), 1.17 (3 H, t, *J* 7 Hz, CH_3CH_2N), 3.50 (2 H, *J* 7 Hz, q, CH_3CH_2N), 3.69, 4.03 (4 H, 2 m, 2 × CH_2N), 4.39 (1 H, s, 3-H), 5.19, 5.34 (4 H, 2 × s, 2 × $ArCH_2O$), 5.58 (1 H, s, 5-H), 5.65 (1 H, d, *J* 6.5 Hz, s on D_2O exch., CHNH), 7.30–7.60 (9 H, m, ArH), 7.71, 8.26 (4 H, dd, ArH), 8.17 (1 H, narrow d, s on D_2O exch., NHC*H*O), 8.82, 9.01 (2 H, 2 × br s, D_2O exch., 2 × NH), and 10.00 (1 H, d, *J* 6.5 Hz, D_2O exch., CHN*H*); *m*/*z* (positive FAB; thioglycerol) MH^+, 845. Less polar column fractions on pooling and evaporation gave the corresponding (2*S*)-penicillin ester (0.209 g, 31%), δ[$(CD_3)_2CO$], *inter alia*, 1.35 and 1.41 (6 H, 2 × s, Me_2C).

Sodium 6β-{(2R)-2-(4-*Aminophenyl*)-2-[(4-*ethyl*-2,3-*dioxopiperazin*-1-*yl*)*carbonylamino*]*acetamido*}-6α-*formamidopenicillanate* (**3**).—The (2*R*)-ester (**17**) (0.131 g, 0.155 mmol) in THF (10 ml) was treated with sodium hydrogen carbonate (0.013 g, 0.155 mmol) in water (2 ml), then 10% palladium on charcoal (0.130 g) was added, and the mixture was hydrogenated at ambient temperature and pressure. After 2 h no starting material was visible by TLC; the mixture was filtered, the precipitate was washed with water and THF, and the combined filtrates were washed twice with ethyl acetate. The aqueous phase was again filtered, concentrated to a small volume, and lyophilised to give the title penicillin (**3**) (0.087 g, 94%); ν_{max} 1 771, 1 710sh, 1 675, 1 610, and 1 512 cm^{-1}; δ(D_2O) 0.89, 1.28 (6 H, 2 × s, Me_2C), 1.18 (3 H, t, *J* 7 Hz, CH_3CH_2N), 3.50 (2 H, q, *J* 7 Hz, CH_3CH_2N), 3.68, 3.99 (4 H, 2 × m, 2 × CH_2N), 4.16 (1 H, s, 3-H), 5.34 (1 H, s, 5-H), 5.58 (1 H, s, CHNH), 6.85, 7.30 (4 H, dd, ArH), and 8.11 (1 H, s, NHC*H*O); *m*/*z* (positive FAB; thioglycerol) MH^+, 598 (100%) and MNa^+, 620 (40%).

Study of the Aqueous Degradation of (**3**).—The penicillin (**3**) (0.070 g, 0.12 mmol) in water (0.7 ml) was kept at ambient temperature; the pH of the solution was recorded at intervals as shown in Figure 2, in comparison with an aqueous solution of penicillin (**1**) at the same concentration. After 48 h, when the pH was 5.0, HPLC and NMR analysis indicated about 20% loss of (**3**). After 119 h, NMR and HPLC analysis showed complete loss of (**3**) with formation of a complex mixture of products.

(2R)-2-[3,4-*Bis*(*benzyloxycarbonyloxy*)*phenyl*]-2-[(4-*ethyl*-2,3-*dioxopiperazin*-1-*yl*)*carbonylamino*]*acetic Acid* (**19**).—(2*R*)-2-(3,4-Dihydroxyphenyl)-2-[(4-ethyl-2,3-dioxopiperazin-1-yl)-carbonylamino]acetic acid (**18**)[1] (12.64 g, 36 mmol) in water (100 ml) and THF (50 ml) was basified to pH 8.0 using 20% aqueous sodium carbonate. Benzyl chloroformate (10.2 ml, 72 mmol) was added dropwise with vigorous stirring, maintaining a pH of 8.0 (using the same base) and a temperature of 25–30 °C. When the pH was stationary for 0.25 h, ethyl acetate (75 ml) was added and the aqueous phase acidified to pH 2.0 using 2M hydrochloric acid. The organic phase was separated and the aqueous phase further extracted with ethyl acetate (30 ml), then the organic phases were combined, washed with water (3 × 50 ml), and evaporated. The resulting oil was dissolved in CH_2Cl_2 (20 ml); the solution was left overnight at 5 °C, and then the crystals were filtered off and dried to give the *protected acid* (**19**) (15.0 g, 81%), m.p. 82–86 °C (Found: C, 60.1; H, 4.6; N, 6.5. $C_{13}H_{29}N_3O_{11}$ requires C, 60.1; H, 4.7; N, 6.8%); ν_{max} 1 773, 1 741, 1 719, 1 688, 1 669, and 1 505 cm^{-1}; δ[$(CD_3)_2SO$] 1.08 (3 H, t, *J* 7 Hz, CH_3CH_2N), 3.40 (2 H, q, *J* 7 Hz, CH_3CH_2N), 3.55, 3.90 (4 H, 2 × m, 2 × CH_2N), 5.25 (4 H, s, 2 × $PhCH_2O$), 5.45 (1 H, d, *J* 6 Hz, CHNH), 7.40–7.55 (13 H, m, ArH), and 9.92 (1 H, d, *J* 6 Hz, CHN*H*); *m*/*z* (positive FAB; thioglycerol) MH^+, 620.

Benzyl 6β-{(2R)-2-[3,4-*Bis*(*benzyloxycarbonyloxy*)*phenyl*]-2-[(4-*ethyl*-2,3-*dioxopiperazin*-1-*yl*)*carbonylamino*]}-6α-*formamidopenicillanate* (**20**).—The protected acid (**19**) (6.19 g, 10 mmol) and benzyl 6β-amino-6α-methylthiopenicillanate (**16**)[12] (3.87 g, 11 mol) were dissolved in CH_2Cl_2 (100 ml) and stirred at 5 °C. *N*,*N*′-Dicyclohexylcarbodi-imide (2.08 g, 10 mmol) in CH_2Cl_2 (20 ml) was added dropwise over 0.25 h at the same

temperature, then stirring was continued for 2.5 h while the mixture was allowed to regain ambient temperature. The precipitate was filtered off and the filtrate washed sequentially with 1M hydrochloric acid (3 × 50 ml) and water (50 ml). The dried extract was finally concentrated to *ca.* 30 ml and added dropwise to a vigorously stirred excess of ether (300 ml). The precipitated white solid was filtered off, washed with ether, and dried to give 9.5 g of material which without further purification was dissolved in a mixture of DMF (10 ml), ethyl acetate (35 ml), and pyridine (10 ml). The solution was stirred, warmed to 30 °C and treated with *N,N*-bis(trimethylsilyl)formamide (8.40 ml, 40 mmol), then with powdered copper(I) chloride (1.50 g, 15 mmol). After being stirred at 30 °C for 3 h, the mixture was cooled to ambient temperature and filtered, the residue being washed with further ethyl acetate (25 ml). The combined filtrates were washed sequentially with 2M hydrochloric acid (2 × 60 ml) and 50% saturated aqueous sodium chloride (2 × 60 ml). Evaporation gave a pale brown syrup which was crystallised by dissolution in ethanol (50 ml) and stirring at 5 °C for 2 h. The solid was filtered, washed with ethanol containing 10% ethyl acetate, and dried to afford the penicillin ester (**20**) (4.1 g, 43%); ν_{max} 1 774, 1 720sh, 1 700sh, 1 675, and 1 498 cm^{-1}; δ[$(CD_3)_2SO$] 0.80, 1.07 (6 H, 2 × s, Me_2C), 1.08 (3 H, t, *J* 7 Hz, CH_3CH_2N), 3.45 (2 H, q, *J* 7 Hz, CH_3CH_2N), 3.54, 3.88 (4 H, 2 × m, 2 × CH_2N), 4.46 (1 H, s, 3-H), 5.16, 5.23 (6 H, 2 × s, 3 × $PhCH_2O$), 5.45 (1 H, s, 5-H), 5.64 (1 H, d, *J* 7 Hz, s on D_2O exch., CHNH), 7.30–7.50 (18 H, m, ArH), 8.04 (1 H, narrow d, *J* 1 Hz, s on D_2O exch., NHCHO), 9.20 (1 H, narrow d, *J* 1 Hz, D_2O exch., NHCHO), 10.02, (1 H, d, *J* 7 Hz, D_2O exch., CHNH), and 10.09 (1 H, br s, D_2O exch., C-6-NH); *m/z* (positive FAB; thioglycerol) MH^+, 951.

(3S,7S,7aR)-5-{(2R)-2-(3,4-*Dihydroxyphenyl*)-2-[(4-*ethyl*-2,3-*dioxopiperazin*-1-*yl*)*carbonylamino*]*acetamido*}-7-*formamido*-2,2-*dimethyl*-2,3,7,7a-*tetrahydroimidazo*[5,1-b]*thiazole*-3,7-*dicarboxylic Acid* (**21**).—The penicillin ester (**20**) (25.0 g, 26.3 mmol) was dissolved in THF (250 ml) and treated with 2M hydrochloric acid (50 ml) at ambient temperature for 1 h. After dilution with ethyl acetate (200 ml), the organic phase was separated and evaporated to give a foam. The latter was dissolved in methanol (250 ml) and subjected to hydrogenation at ambient temperature and pressure in the presence of 50% aqueous 10% palladium on charcoal (6.0 g). The catalyst was then filtered off and washed with methanol, and the combined filtrates were evaporated to give a foam which was redissolved in methanol (30 ml), filtered, and reprecipitated by addition of ether (300 ml) to give the *penillic acid* (**21**) as a very hygroscopic solid (11.1 g, 71%); ν_{max} 1 710, 1 670, 1 611, 1 508, and 1 460 cm^{-1}; δ[$(CD_3)_2SO$], *inter alia*, 1.08 (3 H, t, *J* 7 Hz, CH_3CH_2N), 1.36, 1.40 (6 H, 2 × s, Me_2C), 3.39 (2 H, q, *J* 7 Hz, CH_3CH_2N), 3.55, 3.90 (4 H, 2 × m, 2 × CH_2N), 4.57 (1 H, s, 3-H), 5.35 (1 H, d, *J* 7 Hz, CHNH), 5.64 (1 H, br s, 7a-H), 6.60–6.80 (3 H, m, ArH), 7.10–7.60 (1 H, m, s on D_2O exch., NHCHO), and 9.69 (1 H, d, *J* 7 Hz, CHNH) (Found: MH^+, 593.170. $C_{24}H_{29}N_6O_{10}S$ requires MH^+, 593.167).

(2R,4S)-2-[{(2R)-2-(3,4-*Dihydroxyphenyl*)-2-[(4-*ethyl*-2,3-*dioxopiperazin*-1-*yl*)*carbonylamino*]*acetamido*}(*carboxy*)*formamidomethyl*]-5,5-*dimethylthiazolidine*-4-*carboxylic Acid Disodium Salt* (**22**).—The penillic acid (**21**) (13.5 g, 22.8 mmol) was dissolved in a mixture of water (300 ml) and THF (50 ml) and treated with saturated aqueous sodium hydrogen carbonate to pH 8.0 at ambient temperature. After 0.5 h, organic solvent was removed *in vacuo* and the red aqueous solution chromatographed on HP20SS resin ('Diaion'), with water as eluant. Appropriate fractions were pooled and lyophilised to give the disodium penicilloate (**22**) (8.05 g, 54%); ν_{max} 1 709, 1 675, 1 610sh, 1 506, and 1 457 cm^{-1}; δ(D_2O) 1.16 (3 H, t, *J* 7 Hz, CH_3CH_2N), 1.18, 1.46 (6 H, 2 × s, Me_2C), 3.11 (1 H, s, 4-H), 3.48 (2 H, q, *J* 7 Hz, CH_3CH_2N), 3.64, 3.96 (4 H, 2 × m, 2 × CH_2N), 4.97 (1 H, s, 2-H), 5.37 (1 H, s, CHNH), 6.80–6.95 (3 H, m, ArH), and 7.25 (1 H, s, NHCHO); *m/z* (positive FAB; thioglycerol) MH^+, 655 and $M - Na + H^+$, 633.

6β-[(2R)-2-(3,4-*Dihydroxyphenyl*)-2-{N′-([2-(N-*oxaloethylamino*)*ethyl*]*ureido*}*acetamido*]-6α-*formamidopenicillanic Acid* (**23**).—The penicillin sodium salt (**1**) (30.7 g, 50 mmol) in water (45 ml) at 5 °C was treated with 2M sodium hydroxide to pH 10.7 and set aside for 0.2 h. After neutralisation with 2M hydrochloric acid to pH 7.0 the solution was chromatographed on HP20SS resin, with water as eluant. Appropriate fractions were pooled and lyophilised to give the title penicillin (**23**) as a hygroscopic solid (7.0 g, 22%); ν_{max} 1 770, 1 670sh, 1 608, and 1 526 cm^{-1}; δ(400 MHz; D_2O) 0.97, 0.98, 1.36 (6 H, 3 × s, Me_2C), 1.10–1.25 (3 H, m, CH_3CH_2N), 3.30–3.50 (6 H, m, 3 × CH_2N), 4.23 (1 H, s, 3-H), 5.19, 5.21 (1 H, 2 × s, CHNH), 5.65 (1 H, s, 5-H), 6.96, 7.04 (3 H, 2 × s, ArH), and 8.19 (1 H, s, NHCHO); the preceding spectrum is additionally complicated by rotational isomerism in the oxamate side chain; *m/z* (positive FAB; thioglycerol) MH^+, 655, and MNa^+, 677.

Benzyl (2R,4S)-5,5-*Dimethyl*-2-{R-[(2,2,2-*trichloroethoxy*)*carbonylamino*](*formamido*)(*methoxycarbonyl*)*methyl*}*thiazolidine*-4-*carboxylate* (**26**).—Benzyl 6α-formamido-6β-[(2,2,2-trichloroethoxy)carbonylamino]penicillanate (**24**) (1.50 g, 2.90 mmol) in CH_2Cl_2 (15 ml) and methanol (2 ml) was stirred with triethylamine (0.35 ml) at ambient temperature for 18 h. The mixture was diluted with ethyl acetate, evaporated to dryness, and chromatographed on silica gel, with ethyl acetate–hexane mixtures as eluant. Appropriate fractions containing material less polar than (**24**) were pooled and evaporated to give the penicilloate (**26**) (0.216 g, 14%) as a foam; ν_{max}(CH_2Cl_2) 1 735 and 1 690 cm^{-1}; δ($CDCl_3$) 1.14, 1.50 (6 H, 2 × s, Me_2C), 3.70–3.95 (4 H, m, CH_3O + 4 − H), 4.60–4.95 (2 H, q, Cl_3CCH_2O), 5.19 (2 H, s, $PhCH_2O$), 5.60 (1 H, br s, sharpened on D_2O exch., 2-H), 6.80–6.95 (1 H, m, OCONH), 7.30–7.50 (6 H, m, ArH + NH), and 8.16 (1 H, s, NHCHO); *m/z* (positive FAB; Carbowax) MH^+, 556.

Benzyl (2R,4S)-2-[R-(*Amino*)(*formamido*)(*methoxycarbonyl*)*methyl*]-5,5-*dimethylthiazolidine*-4-*carboxylate* (**25**).—Benzyl 6α-formamido-6β-aminopenicillanate (**15**) (0.200 g, 0.57 mmol) in CH_2Cl_2 (1 ml) and methanol (1 ml) was treated with triethylamine (0.5 ml) and stirred at ambient temperature. After 17 h the solution was evaporated to dryness and co-evaporated four times with a little chloroform. Chromatography on silica gel, with 2.5% methanol–chloroform as eluant, afforded the penicilloate (**25**) (0.155 g, 71%) as a foam; ν_{max} 1 735, 1 676, 1 585w, 1 497, and 1 453 cm^{-1}; δ[$(CD_3)_2CO$] 1.19, 1.57 (6 H, 2 × s, Me_2C), 3.67 (3 H, s, CH_3O), 3.95–4.15 (1 H, m, D_2O exch., N^3-H), 4.23 (1 H, d, s on D_2O exch., 4-H), 5.10–5.30 (3 H, m, $PhCH_2O$ + 2-H), 7.35–7.50 (5 H, m, ArH), 7.75 (1 H, br s, D_2O exch., NH), and 8.00 (1 H, narrow d, s on D_2O exch., NHCHO); *m/z* (positive FAB; thioglycerol) MH^+, 382.

Benzyl (4S)-4,5-*Dihydro*-5,5-*dimethylthiazole*-4-*carboxylate* (**27**) *and Benzyl* (2S)-2-*Formamido*-3-*mercapto*-3-*methylbutanoate* (**11**).—The penicillin ester (**24**) (0.220 g, 0.42 mmol) in CH_2Cl_2 (10 ml) was stirred at ambient temperature for 96 h with triethylamine (0.1 ml). The final solution was diluted with ethyl acetate, washed with water, and evaporated to dryness. Chromatography on silica gel, with ethyl acetate–hexane mixtures as eluant, gave the *dihydrothiazole* (**27**) (0.048 g, 46%) (Found: *M*, 249.0819. $C_{13}H_{15}NO_2S$ requires *M*, 249.0824); ν_{max} (CH_2Cl_2) 1 745 and 1 690 cm^{-1}; δ($CDCl_3$) 1.30, 1.70 (6 H, 2 × s, Me_2C), 4.64 (1 H, d, 4-H), 5.26 (2 H, m, $PhCH_2O$),

7.30–7.50 (5 H, m, ArH), and 8.13 (1 H, d, 2-H). This partially crystalline material could not be satisfactorily recrystallised owing to its great tendency to hydrolysis; after three weeks at ambient temperature it had been quantitatively hydrolysed to the more polar *penicillamine ester* (**11**), m.p. 83–84.5 °C (from ether–hexane) (Found: C, 58.4; H, 6.3; N, 5.3. $C_{13}H_{12}NO_3S$ requires C, 58.4; H, 6.4; N, 5.2%); ν_{max} 1 735, 1 650, and 1 535 cm^{-1}; δ($CDCl_3$) 1.36, 1.47 (6 H, 2 × *s*, Me_2C), 1.91 (1 H, s, SH), 4.77 (1 H, d, *J* 9 Hz, C*H*NH), 5.20 (2 H, q, PhCH_2O), 6.54 (1 H, d, *J* 9 Hz, NH), 7.38 (5 H, s, ArH), and 8.29 (1 H, s, CHO); *m/z* (chemical ionisation; NH_3) MH^+, 268 and MNH_4^+, 285.

Treatment of Benzyl 6β-*Amino*-6α-*formamidopenicillanate* (**15**) *with Anhydrous Base*.—The penicillin ester (**15**) (0.200 g, 0.57 mmol) in CH_2Cl_2 (2 ml) was stirred at ambient temperature for 7 days with triethylamine (0.5 ml). Work-up and chromatography as described for (**24**) gave the unchanged ester (**15**) (0.143 g), by TLC and NMR comparison, as the only well defined product.

Acknowledgements

We are grateful to Dr. J. H. C. Nayler, Dr. R. J. Ponsford, Dr. R. Southgate, and Mr. A. E. Bird for encouragement and advice during the course of this work and the preparation of the manuscript.

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Paper 9/02521I
Received 15*th June* 1989
Accepted 23*rd September* 1989