Degradation of BRL 36650, a 6α-Formamido Penicillin: C(5)–C(6) Bond Cleavage

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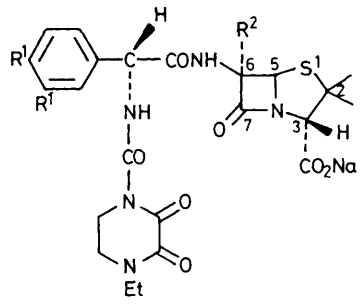
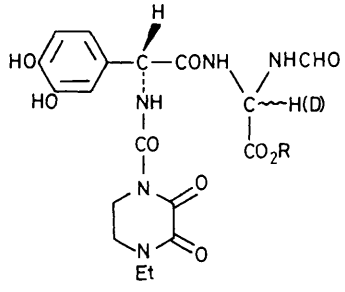
Under mildly acidic aqueous conditions, a 6α-formamido penicillin has been found to degrade with cleavage of the C(5)–C(6) bond as the principal route; more extreme acidic or basic conditions gave other degradation products, which were also characterised.

We recently reported[1] the synthesis of 6α (or 7α)-formamido penicillins and cephalosporins such as BRL 36650 (**1**), novel antibacterial agents with potent activity against Gram-negative bacteria.[2] Subsequently, there have been further reports concerning 7α-formamido cephalosporins ('chitinovorins' and 'cephabacins') isolated from culture filtrates.[3] Penicillins of this structural type show good stability as dry solids, and buffered aqueous solutions kept at low temperature degrade only very slowly; thus an aqueous solution of (**1**) at pH 7 and a concentration of 50 μg ml^{-1} maintained 86% of its potency after 90 days at −20 °C.[4]

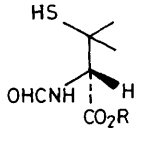
BRL 36650 (**1**) in unbuffered aqueous solution, however, showed some instability, at an increasing rate as the solution became more acidic, as shown by h.p.l.c. and n.m.r. data. Under these conditions we detected (h.p.l.c.) only a small (*ca.* 5%) amount of penicilloic acid; the main reaction pathway



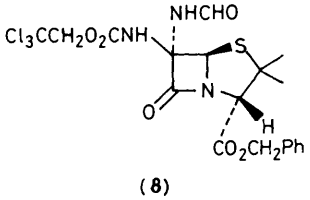
(1) R^1 = OH, R^2 = NHCHO
(2) R^1 = R^2 = H



(3) R = $CHPh_2$
(4) R = H



(5) R = $CHPh_2$
(6) R = H
(7) R = CH_2Ph



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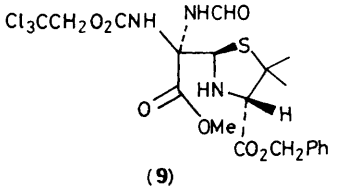
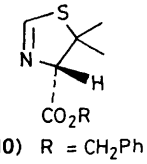


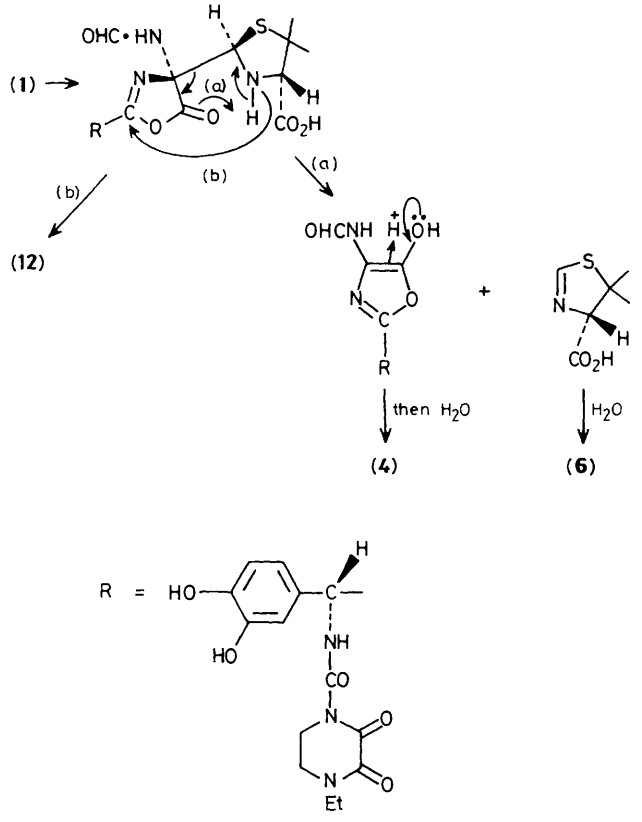
(9)



(10) R = CH_2Ph
(11) R = H

involved cleavage of the C(5)–C(6) bond to give two fragments, in complete contrast to the behaviour of piperacillin[5] (2) and other 6α-H penicillins with an amino or acylamino side chain.

A 0.05 M aqueous solution of (1), initially at pH 6.2, appeared to be completely degraded after 36 h at 25 °C (h.p.l.c.), the final pH being 2.5. Extraction of acidic material and reaction with diphenyldiazomethane gave a mixture of esters, which was separated by silica-gel chromatography. The more polar product was the diastereoisomeric mixture of



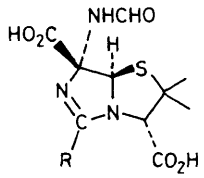
Scheme 1. Degradation pathways of (1).

esters (3),† an approximately 1 : 1 (*R*,*S*) mixture,‡ and the less polar a crystalline material, m.p. 93—94 °C, whose spectral data were those of the *N*-formyl penicillamine ester (5). The latter was independently synthesised by formylation of commercial D-penicillamine, followed by esterification, which gave a product of identical m.p. and rotation.

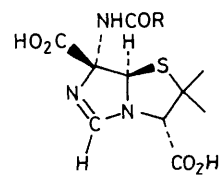
The course of this degradation was monitored by recording the n.m.r. spectra of a solution of (1) in D_2O, at a similar concentration, over 36 h. The rate acceleration of the process was shown by the fact that there was less than 20% degradation after 12 h, but *ca.* 80% after 26 h. After 36 h the cleavage to (4) plus (6) was virtually complete, as indicated in particular by disappearance of the signal at δ 8.10 (1 H, s, NHC*H*O) in the original penicillin. None of the recorded spectra showed more than small amounts of any compounds other than (1), (4), and (6). The chiral carbon atom in (4), which was C(6) in the original penicillin, bears a deuterium atom in the n.m.r. experiment; this is consistent with, though not conclusive evidence for, the mechanism shown in Scheme

† Satisfactory analytical and/or spectroscopic data were obtained for all new compounds.

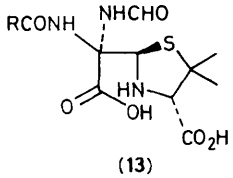
‡ δ[$(CD_3)_2CO$] 1.17 (3 H, t, *Me*CH_2N), 3.49, 3.67, and 4.03 (6 H, 3 m, 3 × CH_2N), 5.46 (1 H, d, C*H*NH), 6.00—6.15 (1 H, m, dd on brief D_2O exchange, NHC*H*NHCHO), 6.70—6.90 (3 H, m, Ar H), 7.01 and 7.02 (1 H, 2 s, $OCHPh_2$), 7.20—7.60 (10 H, m, Ar H), 8.17 (1 H, s on D_2O exchange, NHC*H*O), 8.35, 8.70, and 9.83 (3 H, 3 m, 3 × NH).



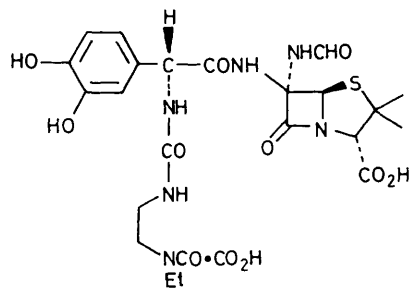
(12)



(12')



(13)



(14)

1, pathway (a). The initial oxazolone formation appears to involve the 6β-acylamino side chain rather than the 6α-formamido substituent, as shown by the structure of the penillic acid (**12**) obtained in acid solution [see below: pathway (b)].

From the abovementioned aqueous conditions and subsequent extractive work-up, a penicilloic acid was not isolated. However, a penicilloate ester could be obtained under anhydrous conditions from the (2,2,2-trichloroethoxy)-carbonyl protected penicillin (**8**).[1] When this material was treated with triethylamine in methanol at ambient temperature for 18 h, virtually complete conversion into the methyl penicilloate (**9**) was observed.§ In the absence of methanol, the degradation proceeded further and the thiazoline (**10**) was isolable with care. This material parallels a known[6] degradation product of benzylpenicillin methyl ester in trifluoroacetic acid; in the presence of water it is very readily hydrolysed to the penicillamine ester (**7**), m.p. 83—84 °C. The C(5)—C(6) bond cleavage is not observed as the major pathway, however, when (**1**) is subjected to more extreme acidic or basic conditions. Thus brief treatment of (**1**) with 0.5 M HCl gave rapid and almost exclusive conversion into the penillic acid (**12**);¶ the latter at pH 7 was transformed to the penicilloic acid (**13**). At intermediate acidic pH (**1**) gave a mixture of (**12**), (**13**), and the cleavage products (**4**) and (**6**). The great ease of base-catalysed opening of the 2,3-dioxopiperazine ring already known from piperacillin (**2**)[7] was again illustrated by the rapid production of (**14**) from (**1**) at pH 10.

N-Formyl-D-penicillamine (**6**) has previously been reported as one of the degradation products of both benzylpenicillin[8] and methicillin[9] in aqueous solution, but no indication of the extent of its formation was given. It also arises from cleavage of benzylpenicillin with carboxypeptidases.[10] It is not certain whether the thiazoline (**11**) is an intermediate in such cases.[11,12] In these laboratories we found that 2% aqueous solutions of benzylpenicillin and methicillin gave respectively about 15 and 50% of *N*-formyl-D-penicillamine after 7 days at 37 °C,[13] in contrast to the nearly complete conversion of (**1**). On the other hand, piperacillin (**2**)[5] in mildly acidic aqueous solution gives, slowly, a mixture of the derived penicilloic and penilloic acids.[4]

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§ δ($CDCl_3$) 1.15, 1.51 (6 H, 2 s, Me_2C), 3.86 (3 H, s, CH_3O), 3.81 (1 H, s, 3-H), 4.66 (2 H, ABq, Cl_3CCH_2O), 5.18 (2 H, ABq, $PhCH_2O$), 5.60 (1 H, br.s, 5-H), 6.88 (2 H, br s, 2 × NH), 7.20—7.44 (6 H, m, ArH + NH), and 8.17 (1 H, s, NHC*H*O). Rotational isomerism about the formamido group was observed.

¶ The alternative penillic acid structure (**12'**) may be ruled out since the n.m.r. spectrum of (**12**) (in C_5D_5N + H_2O or D_2O) shows an –NHCH– unit corresponding to an intact formamido group.

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