The Isolation and Characterisation of (3*R*,5*R*)- and (3*S*,5*R*)-Carbapenam-3-carboxylic Acid from *Serratia* and *Erwinia* Species and their Putative Biosynthetic Role

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Two new β-lactams (**4b**) and (**5b**), (3*R*,5*R*)- and (3*S*,5*R*)-carbapenam-3-carboxylic acid, have been isolated from strains of *Serratia* and *Erwinia* spp.; their possible role, and that of (5*R*)-carbapen-2-em-3-carboxylic acid as intermediates in the biosynthesis of the more complex members of the carbapenem family of β-lactam antibiotics is discussed.

The β-lactam antibiotics thienamycins (**1**) and olivanic acids (**2**) which possess the carbapenem ring system are representative of a substantial family of naturally occurring compounds.[1] These have mainly been isolated from *Streptomyces* species and to date biosynthetic studies on this important group of antibiotics have been restricted by the complexity, low yield, and relative instability of the carbapenems produced. Radioactive and stable isotope studies with *Streptomyces cattleya* have demonstrated that, in thienamycin (**1a**), the C-6 and C-7 of the β-lactam ring are derived from acetate, the cysteaminyl side-chain from cysteine,[2] and both carbon atoms of the hydroxyethyl substituent at C-6 from the methyl of methionine.[2,3] An earlier preliminary account[4] suggested that glutamate was the source of the carbon atoms of the proline moiety. On the basis of these observations it has been proposed that the parent ring system (**3**) is formed initially from the interaction of an acetate unit with a γ-activated form of glutamate and is the precursor of all the carbapenems.[2] The sequence of events leading to the introduction of the substituents at C-2 and C-6 remains somewhat obscure, although the Michael-type addition of thiol derivatives at C-2 and subsequent oxidation has been implicated.[2,5,6] The parent compound, sodium carbapen-2-em-3-carboxylate (**3a**), has been isolated from species of the bacteria *Erwinia* and *Serratia* as its *p*-nitrobenzyl ester (**3b**),[7] and has recently been shown to possess the same absolute configuration as the products from *Streptomyces*.[8]

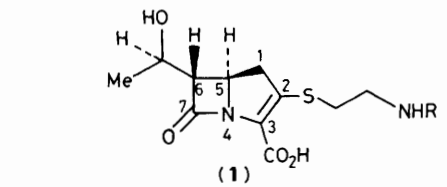
Further to our interest in the biosynthesis of the olivanic acids[5] and other non-traditional β-lactams,[9] we considered it important to establish that the parent system was indeed glutamate derived. The production of (**3a**) by *Serratia* sp ATCC 39006 was demonstrated by means of a β-lactamase induction assay procedure[10] and monitored by h.p.l.c. using an authentic sample of synthetic racemic material[11] as a standard. The susceptibility of (**3a**) to the β-lactamase II from *Bacillus cereus* provided a valuable additional assay procedure.

L-[U-^{14}C]-Glutamate was fed to growing cells of the culture

and after 24 h culture supernatant was ion-pair extracted (*via* Aliquat 336[12] in CH_2Cl_2) with back extraction into sodium iodide solution. The back extract was examined by h.p.l.c. monitored at 260 nm and fractions (taken at 15 s intervals) were assayed for radioactivity to measure uptake of label into (**3a**). Figure 1a shows the plots of radioactivity against the fractions and the u.v. absorption trace; Figure 1b shows the same plots but after treatment of the back extract with β-lactamase II. From these results it was apparent that glutamate was incorporated into (**3a**).† In addition it was evident that a compound labelled with glutamate, and with a similar retention time, but lacking significant absorption at 260 nm was also present. Furthermore, this compound was stable to β-lactamase II and was inactive in antibacterial assays.

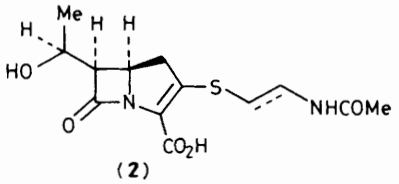
Ion-pair extraction of the culture filtrate, followed by derivatisation with *p*-nitrobenzyl bromide and careful chromatography, afforded the crystalline *p*-nitrobenzyl ester (**3b**), together with two new isomeric esters possessing molecular formulae two mass units greater than (**3b**), in the relative ratio of approximately 9 : 1 [(**4a**)-(**5a**)]. Their i.r. [$\nu_{max.}$ (KBr) 1760 cm^{-1} (β-lactam CO)] and electron impact mass spectra were indicative of β-lactams, and on the basis of their n.m.r. data‡ and a chemical correlation with (**3b**) (see below), the major isomer was identified as the carbapenam (**4a**) and the minor isomer as the C-3 epimer (**5a**). The assignment of the relative stereochemistry at C-3 followed from a detailed analysis of the high field n.m.r. data of (**4a**) and (**5a**). The C-3 α- and β-protons have characteristic chemical shifts[11,13] (δ 4.48 and 3.97 respectively), and the 3α-proton exhibits a distinctive through space coupling to the C-6α proton ($J_{3\alpha,6\alpha}$ 1.3 Hz).[14]

Catalytic hydrogenation of the natural carbapenem ester (**3b**) of known absolute configuration using Pd/C as catalyst under controlled conditions afforded (**5a**) and (**4a**) in the ratio of approximately 9 : 1 (the reverse of that obtained from the natural source). The c.d. spectra of the two samples of (**5a**), *i.e.* derived from the natural source and by hydrogenation of (**3b**), showed identical positive Cotton effects at 230 nm, establishing the full absolute configuration for (**5a**). Insufficient (**4a**) was isolated from the hydrogenation experiment for direct chiral comparison with the ester of the natural material. However the c.d. spectrum of natural (**4a**) displayed a negative Cotton effect at 230 nm and a mirror image

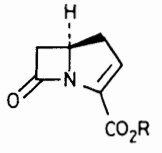


(**1**)

a; R = H

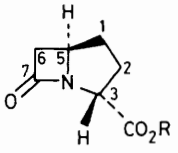


(**2**)



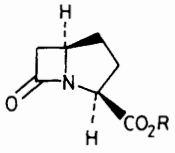
(**3**)

a; R = Na
b; R = PNB



(**4**)

a; R = PNB
b; R = Na



(**5**)

a; R = PNB
b; R = Na

PNB = p-$O_2NC_6H_4CH_2$

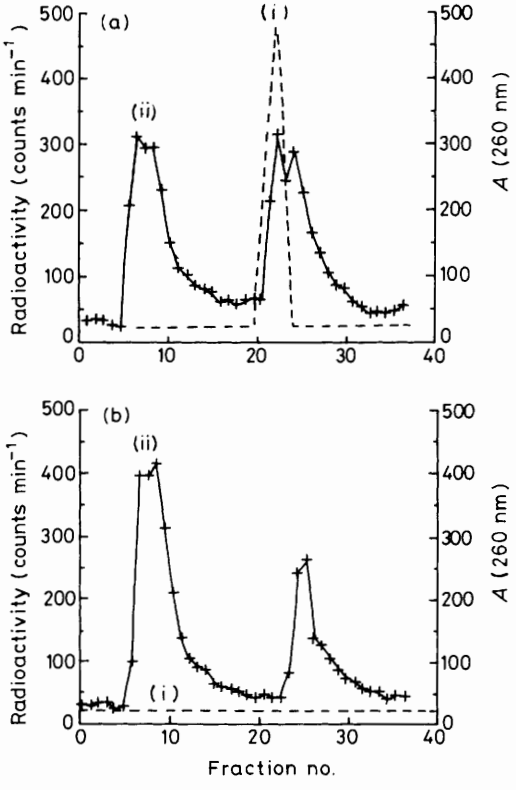


Figure 1. (a) Material from ion-pair extraction of the *Serratia* sp. ATCC 39006 culture filtrate. (b) The same material after treatment with *Bacillus cereus* β-lactamase II. (i) Absorption at 260 nm (arbitrary units); (ii) Plot of radioactivity against fractions taken every 15 s. H.p.l.c. was carried out using a C_{18} reverse phase column eluting with 0.1 M NaH_2PO_4 buffer pH 4 at 2 ml/min and showed a single peak for (**3a**) with a retention time of 310 s.

† Preliminary experiments established that in a defined medium the antibiotic (**3a**) was produced in parallel with growth. The [U-^{14}C]-glutamate (specific activity 50 715 dpm/μmol) was therefore added at the time of inoculation affording the carbapenem [(**3a**), 41 283 dpm/μmol]. The molar specific activity (0.81) of (**3a**) suggested that the label was essentially incorporated intact.

‡ (**3b**) ^{1}H n.m.r. δ (400 MHz, $CDCl_3$, Me_4Si) 2.79 (1 H, ddd, *J* 19.3, 8.1, and 2.6 Hz, 1β-H), 2.97 (1 H, dddd, *J* 19.3, 9.1, 3.2, and 0.8 Hz, 1α-H), 3.01 (1 H, dd, *J* 16.9 and 3.2 Hz, 6β-H), 3.53 (1 H, ddd, *J* 16.9, 5.7, and 0.8 Hz, 6α-H), 4.31 (1 H, dddd, *J* 9.1, 8.1, 5.7, and 3.2 Hz, 5α-H), 5.28 and 5.43 (2 H, ABq, *J* 13.7 Hz, CH_2Ar), 6.60 (1 H, dd, *J* 3.2 and 2.6 Hz, 2-H), 7.61 (2 H, *J* 8.8 Hz, Ar), 8.26 (2 H, *J* 8.8 Hz, Ar). (**4a**) ^{1}H n.m.r. δ (400 MHz, $CDCl_3$, Me_4Si) 1.56 (1 H, m, 1β-H), 2.31 (1 H, m, 1α-H), 2.28 (1 H, m, 2α-H), 2.60 (1 H, m, 2β-H), 2.67 (1 H, dd, *J* 15.8, and 2.1 Hz, 6β-H), 3.31 (1 H, dd, *J* 15.8 and 4.9 Hz, 6α-H), 3.88 (1 H, dddd, *J* 10, 5.4, 4.9, 2.1 Hz, 5α-H), 4.48 (1 H, dd, *J* 7.6 and 7.6 Hz, 3β-H), 5.52 (2 H, s, CH_2Ar), 7.52 (2 H, d, *J* 8.9 Hz, Ar), 8.24 (2H, d, *J* 8.9 Hz, Ar). (**5a**) ^{1}H m.n.r. δ (400 MHz, $CDCl_3$, Me_4Si) 1.81 (1 H, m, 1β-H), 2.16 (1 H, m, 1α-H), 2.38 (2 H, m, 2α + 2β-H), 2.76 (1 H, dd, *J* 15.9 and 2.2 Hz, 6β-H), 3.14 (1 H, ddd, *J* 15.9, 4.5, and 1.3 Hz, 6α-H), 3.73 (1 H, dddd, *J* 9.5, 5.6, 4.5 and 2.2 Hz, 5α-H), 3.97 (1 H, ddd, *J* 6.3, 3.5, and 1.3 Hz, 3α-H), 5.25 and 5.32 (2 H, ABq, *J* 13.3 Hz, CH_2Ar), 7.56 (2 H, d, *J* 8.9 Hz, Ar), 8.24 (2 H, d, *J* 8.9 Hz, Ar).

relationship with that of (**5a**), strongly supporting the shown epimeric configuration at C-3.

Both (**4b**) and (**5b**) have also been detected in *Erwinia* species that produces (**3a**). It is possible that the dihydro-derivatives (**4b**) and (**5b**) result biosynthetically from the reduction of the carbapenem (**3a**), which in turn could be derived from acetyl coenzyme A and a γ-activated glutamate along the lines already proposed.[2] Alternatively the carbapenams could be derived by condensation of glutamate semi-aldehyde with acetate or an appropriately activated unit. Experiments to distinguish between these two pathways are currently in progress.

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