Two Novel Arginine Derivatives from a Mutant of *Streptomyces clavuligerus*

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Two novel arginine derivatives, N^2-(2-carboxyethyl)arginine and N^2-(2-carboxyethyl)-3-hydroxyarginine, are produced by a mutant of *Streptomyces clavuligerus* dclH 65, which is blocked in clavulanic acid biosynthesis, and the structures of the compounds indicate that they may be involved in clavulanic acid biosynthesis.

In the previous communication,[1] we presented evidence to show that arginine **1** is the amino acid that is processed into the clavulanic acid biosynthetic pathway. The next known intermediate on the pathway is the monocyclic β-lactam proclavaminic acid **2**.[2] Hence, a β-lactam must be elaborated onto the N^2 position of the arginine carbon skeleton, a hydroxy group introduced at C-3 and the N^5 guanidino function hydrolysed, in a yet to be defined sequence. This communication presents results that give an indication of possible biochemical intermediates.

Standard samples of putative intermediates were chemically synthesised.‡ Monocyclic β-lactams **3** and **4** were made by reaction of the parent amines, proclavaminic acid **2**[2] and dehydroxyproclavaminic acid,[2] respectively, with amino-iminomethanesulfonic acid.[3] The β-amino acid (2*S*)-**5** was obtained by the route shown in Scheme 1 and the β-amino acid **6** by acidic hydrolysis of the β-lactam **4**.

None of these putative metabolites was readily detectable in culture supernatant from *S. clavuligerus*. We therefore examined mutants of *S. clavuligerus* that were blocked in the production of proclavaminic and hence clavulanic acid. Some of these mutants accumulated compounds containing an amine group as shown by derivatisation with phenylisocyanate.[4] A selection of such mutants were fermented and the culture broths screened for the presence of compounds bearing guanidino groups by the Sakaguchi colour reaction.[5] The mutant strain *S. clavuligerus* dclH 65 gave a good response to the colour test. Therefore, the culture filtrate was fractionated using Dowex-50, Pharmacia HR5/5 Mono S and Partisil 10SCX cation exchange matrices, the purification of guanidino containing materials being followed by a modified Sakaguchi test procedure.§ Two compounds were isolated in the approximate ratio 10:1 and, by comparison of their spectroscopic properties (^{1}H NMR and FAB MS for both compounds and ^{13}C for the major component) with those of the compounds prepared as speculative intermediates, the structures were assigned as **5** and **6** (major to minor).

Since the chromatographic purification process of the *S. clavuligerus* dclH 65 culture broth involved acidic media, there was a possibility that **5** and **6** were ring-opened artifacts of the corresponding β-lactam derivatives **3** and **4**; although analytical HPLC of fresh culture filtrate did not show the presence of ring closed materials. Therefore, a further fractionation of culture broth was carried out maintaining near neutral conditions to minimise hydrolysis of any β-lactam compounds. Fresh culture filtrates of dclH 65 were centrifuged, freeze dried, treated with ethanol–water (1:1) to remove high molecular mass compounds, and the soluble fraction concentrated. The concentrate was then chromatographed over Amberlite IRA68 anion exchange resin, Sephadex Biogel P2 and silica gel whilst maintaining the pH of eluates between 4 and 7.5. Sakaguchi-positive materials were collected at each stage. The major Sakaguchi-positive material was isolated and analysed by ^{1}H NMR, IR, circular dichroism and mass spectrometry. These data confirmed the structural assignment of the major product as (**S**)-**5**. the minor Sakagu-

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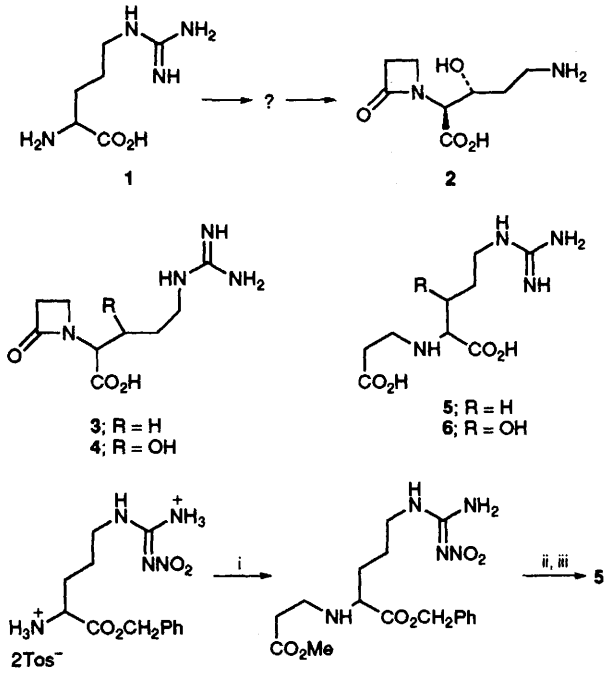
‡ Satisfactory analytical and/or spectroscopic data were obtained for all new compounds.

§ The intensity of the colour formed in the Sakaguchi reaction was measured at 515 nm.



Scheme 1 *Reagents and conditions*: i, Et_3N–methyl acrylate (56%); ii, NaOH–tetrahydrofuran/H_2O (1:1); iii, H_2, Pd-C (10%), (45% overall for ii and iii) (Tos = *p*-$MeC_6H_4SO_3$)

chi-positive compound was not present in sufficient quantity for isolation but a material was detected which co-eluted with synthetic **6** on HPLC and capillary zone electrophoresis.

The arginine derivatives **5** and **6** are members of the opine[6] class of natural products, the closest related members being octopine, N^2-(1-carboxyethyl)arginine[7] and acetopine, N^2-carboxymethylarginine,[8] which occur in the crown galls of plants infected with *Agrobacterium tumefaciens*.[9] Octopine was first isolated from octopus muscle.[7] Although a number of studies have been reported on the metabolic utilisation of opines by microorganisms[10] we are not aware of opines having been isolated as metabolic products from any microbial source other than the *A. tumifaciens* related galls. The nearest analogues from another procaryotic source are (2*S*,7*S*)-N^5-(1-carboxyethyl)ornithine[11] and (2*S*,8*S*)-N^5-(1-carboxyethyl)lysine,[12] produced by *Streptococcus lactis*.

The occurrence of **5** and **6** in *S. clavuligerus* dclH 65 does not in itself prove their involvement in clavulanic acid biosynthesis. However, their structures are obvious candidates as precursors of proclavaminic acid **2** and several logical biosynthetic sequences can be postulated based on their structures. Further experimental data on the possible role of these compounds as intermediates of the clavulanic acid biosynthetic pathway are described in the following communication.

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