N-Acyl Derivatives of Clavaminic Acid Produced by a Mutant of *Streptomyces clavuligerus*

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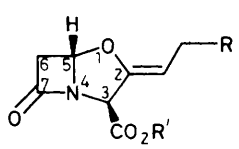
A mutant of *Streptomyces clavuligerus*, blocked in clavulanic acid production, was found to accumulate three *N*-acyl derivatives of the β-lactam clavaminic acid in the culture broth.

In a previous communication we reported the isolation of clavaminic acid (**1**)[1] from *Streptomyces clavuligerus* and presented evidence[2] that this intracellular metabolite, which possesses the 3*S*,5*S* stereochemistry, is a biosynthetic precursor of the β-lactamase inhibitor clavulanic acid (**2**) which possesses the 3*R*,5*R* stereochemistry. We now report the isolation of three *N*-acyl derivatives of (**1**) from the extracellular culture fluid of *S. clavuligerus* dcl-8.

S. clavuligerus dcl-8 is a blocked mutant which produces no clavulanic acid as judged by bioassay[3] but when the extracellular culture broth was reacted with imidazole at room temperature[4] a chromophore (λ_{max} 313 nm) was produced which indicated that clavulanic acid, or a related metabolite, was present. Examination of the imidazole derivatized broth by reverse-phase h.p.l.c.[5] confirmed that clavulanic acid was absent; however, a major peak with a slower retention time was observed. This novel metabolite was isolated as the sodium salt by aqueous column chromatographic methods. A portion of this salt was reacted with *p*-bromobenzyl bromide to give, after purification, a crystalline ester. ^{1}H n.m.r., ^{13}C n.m.r., circular dichroism (c.d.) spectroscopy and mass spectrometry indicated the purified compounds to have structures (**3**) and (**4**). The c.d. spectrum of (**3**) gave a positive Cotton effect at 211 nm and a negative at 235 nm, the same as previously reported for (**1**).[1] The structural assignment was confirmed by the following synthesis.

Sodium 9-aminodeoxyclavulanate (**5**), prepared[6] from clavulanic acid, was refluxed with acetylglycine, dicyclohexylcarbodiimide and sodium carbonate in anhydrous tetrahydrofuran. The purified product (**6**) of this reaction was identical (by n.m.r. and h.p.l.c. analyses) to the natural metabolite (**3**). However, the circular dichroism spectra of the natural and synthetic compounds were opposite, thus confirming that they were enantiomers. The β-lactamase inhibitory activity of the metabolite (**3**) was negligible as was the case with clavaminic acid[1] whereas (**6**) was a potent inhibitor (Table 1) as would be expected for a 3*R*,5*R* clavulanate derivative.



(**1**) R = NH_2 R′ = H
(**3**) R = $NHCOCH_2NHCOMe$ R′ = Na
(**4**) R = $NHCOCH_2NHCOMe$ R′ = CH_2–C$_6$H$_4$–Br
(**7**) R = NHCOMe R′ = CH_2–C$_6$H$_4$–Br
(**8**) R = $NHCOCH_2NH_2$ R′ = H

During the purification of a large batch of (**4**) by silica gel chromatography, a minor, faster eluting component was isolated which was crystallised and shown to possess the structure (**7**). The c.d. spectrum of this compound gave a positive inflection at 219 nm and a negative inflection at 243 nm indicating the 3*S*,5*S* stereochemistry. A third metabolite was isolated in trace amounts from culture broth in the salt form. ^{1}H n.m.r. and fast atom bombardment (f.a.b.) mass spectrometry data were consistent with structure (**8**). A c.d. spectrum was not obtained, but as (**8**) exhibited no β-lactamase activity it is likely that it also possesses the 3*S*,5*S* stereochemistry.

S. clavuligerus dcl-8 produces titres of metabolite (**3**) which are comparable to the titres of clavulanic acid produced by its parent strain. We have been unable to detect the presence of the three novel metabolites in clavulanic acid producing strains. We assume, therefore, that *S. clavuligerus* dcl-8 is blocked in the clavulanic acid biosynthetic pathway between clavaminic acid and clavulanic acid, resulting in the intracellular accumulation of clavaminic acid which is then acylated and excreted into the external medium.

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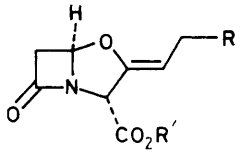

(**2**) R = OH R′ = H
(**5**) R = NH_2 R′ = H
(**6**) R = $NHCOCH_2NHCOMe$ R′ = Na

Table 1. β-Lactamase inhibitory activities of (**3**) and (**6**).

Source of β-lactamase	I_{50}[a]/μg ml^{-1} (**3**)	(**6**)
Staphylococcus aureus Russell	>50	0.05
Escherichia coli K12, RGN 238 (OXA-1)	>50	0.35
Escherichia coli JT4 (TEM-1)	>50	0.08
Proteus mirabilis C889	50	0.05
Klebsiella pneumoniae E70	24	0.04

[a] I_{50} is the concentration of compound required to inhibit the rate of hydrolysis of nitrocefin ([S] = 250 μg ml^{-1}) by 50%. The test compounds were incubated with the enzymes for 5 min before addition of substrate.

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