Note

PRODUCTION OF THE CEPHALOSPORATE C-2 BY A *STREPTOMYCES LACTAMDURANS* MUTANT DEFECTIVE IN CEPHAMYCIN C BIOSYNTHESIS

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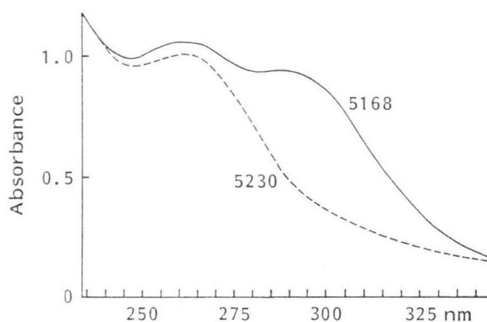
*Streptomyces lactamdurans** produces the β-lactam antibiotic cephamycin C[1]. We report here the isolation of a mutant of *S. lactamdurans* which also produces the unusual fermentation product D-5-amino-5-carboxyvaleramido-(5-formyl-4-carboxy-2*H*,3*H*,6*H*-tetrahydro-1,3-thiazinyl)glycine.

Unlike its parent culture, MA5230, the nitrosoguanidine-induced mutant strain MA5168 produced a compound with an absorbance peak at 300 nm (Fig. 1). In addition, this mutant generated significantly less cephamycin C bioactivity than the parent. The amount of cephamycin C formed was also much lower than was expected based on the serine protease activity of the strain (Table 1).[2] Serine protease has previously been correlated with cephamycin C production. Thus, we suspected that MA5168 might contain a mutation which blocks *de novo* cephamycin C biosynthesis, causing accumulation of a biosynthetic intermediate or shunting intermediates to other products through an alternate pathway.

Fig. 1. UV spectra of final fermentation broths of MA5168 and MA5230.

Samples were centrifuged to remove cells and the supernatant was diluted 1:100 in Tris-HCl buffer (100 mM, pH 7.0) prior to spectral scan.



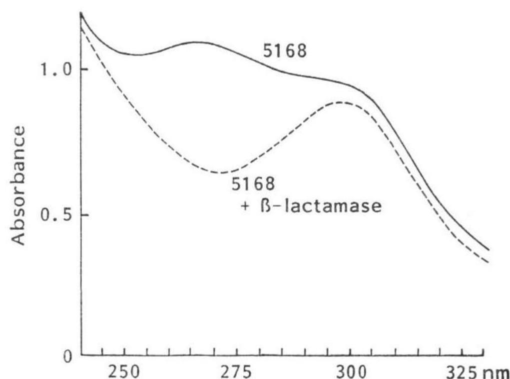
Fermentation broths from MA5168 and MA5230 were treated with *Enterobacter cloacae* β-lactamase to eliminate the contribution of β-lactam compounds from the UV spectrum. The 300 nm peak in these broths was not diminished by this treatment; a result compatible with the absence of a β-lactam ring in the unknown compound (Fig. 2).

Table 1.

Culture	Cephamycin C (units/ml)	Serine protease (units/ml)	Protease/ cephamycin C
MA5230	9.3	106	11
MA5168	2.7	101	37

Fig. 2. UV spectra of final fermentation broth of MA5168 after cephalosporinase treatment.

Centrifuged samples were incubated 30 minutes at room temperature with 1,000 units of *Enterobacter cloacae* cephalosporinase/ml. Samples were then diluted 1:100 in Tris-HCl buffer (100 mM, pH 7.0), and subjected to spectral scan.



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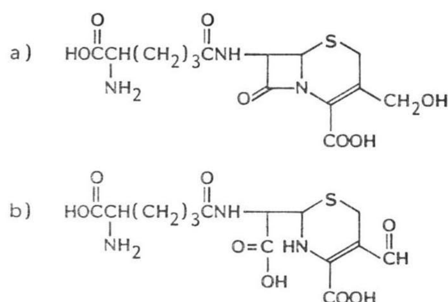
* Preliminary biochemical data indicate that this organism may belong to the genus *Nocardia* (S. A. CURRIE, unpublished data)

Fig. 3. The structures of deacetylcephalosporin C (a) and the cephalosporate C-2 (b).



Throughout isolation from diluted fermentation broth of MA5168 the unknown was determined by its UV absorption while cephamycin C activity was determined by antibacterial bioassays against the tester organism *Vibrio percolans* MB1272.

Final fermentation broth was centrifuged to remove mycelia and the supernatant was adjusted to pH 8.0 prior to filtration through a pad of filter-aid. The filtrate was then absorbed onto 1/7 volume of Dowex 1X2 chloride cycle resin and the column was eluted with 2% NaCl in water. About half of the cephamycin C was found in the broth fractions which came through after loading, and about half was found in the first two column volumes of eluate. Most of the UV absorbance at 300 nm was found in the third column volume of eluate. This fraction was concentrated under reduced pressure, desalted by chromatography on Bio-Gel P-2, and chromatographed on microcrystalline cellulose in the solvent system acetonitrile - 1-propanol - water (2: 2: 1). The 300 nm absorbing compound eluted between 3.5 and 4.5 column volumes of eluate. This fraction was concentrated to dryness under reduced pressure. Analysis by NMR and UV established the structure: D-5-Amino-5-carboxyvaleramido-(5-formyl-4-carboxy-2*H*,3*H*,6*H*-tetrahydro-1,3-thiazinyl)glycine (Fig. 3b). Key NMR features (D_2O) were the presence of typical aminoadipoyl signals; vicinal methine doublets at 4.69 and 4.95 (J=5.9 Hz); a non-equivalent, isolated methylene at 3.53 and 3.58 (J=16.0 Hz); and a singlet at 9.18 (1H) ppm. The UV spectrum showed max (H_2O) 300 nm ($E^{1\%}_{1cm}$ 350).

This cephalosporate has been previously observed by FUJISAWA and KANZAKI[3] in fermentation broths of a mutant of *Cephalosporium acremonium*, the fungus which produces cephalosporin C. Based on studies by HAMILTON-MILLER *et al.*[4] on the cleavage of β-lactam rings of cephalosporins, they suggested that the new cephalosporate, which they designated C-2, was a degradation product of the cephalosporin C precursor, deacetylcephalosporin C (Fig. 3a). The mutants which accumulated high levels of C-2 in *C. acremonium* all had depressed acetyl CoA: deacetylcephalosporin C acetyltransferase activity, the reaction which acetylates deacetylcephalosporin C to cephalosporin C.

By analogy, we expect that MA5168 contains a partial block in a biosynthetic step of cephamycin C biosynthesis, and that this causes accumulation of deacetylcephalosporin C which is then degraded to C-2. Since *S. lactamdurans* does not produce cephalosporin C, the reaction blocked would not be an acetylation, but rather the most likely subsequent step in cephamycin C biosynthesis, carbamoylation[5] of deacetylcephalosporin C.

It has been previously suggested[5] that deacetylcephalosporin C is an intermediate for both cephamycin C and cephalosporin C biosynthesis, and that carbamoylation or methoxylation occur after deacetylcephalosporin C is produced. Our results, showing the production of the cephalosporate C-2 by a streptomycete producing cephamycin C, would be consistent with this hypothesis.

Acknowledgments

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