

OCCURRENCE OF A
3-METHYLTHIOMETHYLCEPHEM
DERIVATIVE IN A CULTURE BROTH
OF *CEPHALOSPORIUM* MUTANT

Sir:

A large number of cephem derivatives have been chemically synthesized. Only a few cephem compounds, however, have been detected in culture fluids of microorganisms, that is, cephalosporin C (CPC) from *Cephalosporium*^{1,2,3)} or *Emericellopsis*¹⁾, deacetylcephalosporin C (DCPC) from *Cephalosporium*^{4,5)} and several 7-methoxycephalosporin compounds from *Streptomyces*^{6,7)}.

This communication is concerned with the identification of a cephem derivative (F-1) which had not previously been demonstrated in any of the microbial cultures.

After a potent CPC-producing mutant, strain No. 155, had been cultivated for six days at 28°C in a medium, an aliquot (0.05 ml) of the broth was subjected to electrophoresis (95 V/cm, 70 min.) in 10% acetic acid (pH 2.2) on a sheet of Whatman No. 1 filter paper (12×42 cm) and F-1 which was not formed by parent strain No. 229 was clearly separated. (Fig. 1). To prepare F-1, a number of aliquots of broth were subjected to the electrophoresis and each F-1 fraction was eluted with a small volume of water. For further purification, this fraction was chromatographed on thin-layer cellulose plates (20×20 cm, 0.1 mm, E. Merck) in *n*-butanol-acetic acid-water (3:1:1,

v/v), eluted again into a small volume of water and then crystallized as Na salt.

Since the infrared spectrum of the purified F-1 showed characteristic bands at 1740 and 1385 cm⁻¹ consistent with β-lactam carbonyl and thiomethyl groups, F-1 was presumed to be similar to 7-(5-amino-5-carboxypentamido)-3-methylthiomethyl-3-cephem-4-carboxylic acid (MTC). Therefore, F-1 was compared with an authentic sample of MTC. The authentic MTC was chemically synthesized by heating a mixture of CPC·Na·2H₂O (5.5 mmoles) and CH₃SH (27 mmoles) in 25 ml water at 70°C for 3 hours⁸⁾.

The R_f values of CPC, DCPC, deacetylcephalosporin C lactone (Cc), F-1 and MTC on a thin-layer cellulose plate in *n*-propanol-water (7:3, v/v) were 0.42, 0.33, 0.36, 0.55 and 0.55, respectively, and those in *n*-butanol-acetic acid-water (3:1:1, v/v) 0.32, 0.19, 0.29, 0.37 and 0.37, respectively. A typical electrophoregram performed by the same procedure as described above showed that CPC, DCPC, Cc, F-1 and MTC moved 7, 10.5, 24, 11.5 and 11.5 cm toward the cathode from the origin, respectively. For further confirmation of F-1, the cephalosporins were subjected to liquid chromatography by using an Aminex A-27 column (0.3×50 cm, Bio-rad Laboratories) equilibrated with 1 M acetate buffer (pH 3.5). After 20 μl of the sample (1 mg/ml each) to be tested was injected, elution was performed with the same acetate buffer under 57 kg/cm² at a flow rate of 0.2 ml per minute. Completely separated sharp peaks were seen with retention times 97, 70, 17, 240 and 240 minutes for CPC, DCPC, Cc, F-1 and MTC. Thus, chromatographical analyses with three systems afforded good evidence for the identity of F-1 with MTC.

The ultraviolet spectra of F-1 and MTC in phosphate buffer solutions (0.1 M, pH 6.5) gave the same patterns characteristic for cephalosporins with a maximum at 264 nm and a minimum at 230 nm. The infrared spectrum of F-1 was also in good agreement with that of MTC. Antibacterial spectra of F-1 and MTC were the same as shown in Table 1. From these data, the unknown compound F-1 was identified as MTC.

Fig. 1. Paper electrophoregram of the culture broths of strain No. 229 and strain No. 155.

Paper electrophoresis on Whatman No. 1 filter paper was performed in 10% (v/v) acetic acid (pH 2.2) at 95 V/cm for 70 minutes. Metabolites were detected by their absorption of ultraviolet light (○) and coloration with ninhydrin (○).

- Sample: (1) Culture broth of strain No. 229
(2) Culture broth of strain No. 155
(3) Authentic cephalosporin C
(4) Authentic deacetylcephalosporin C

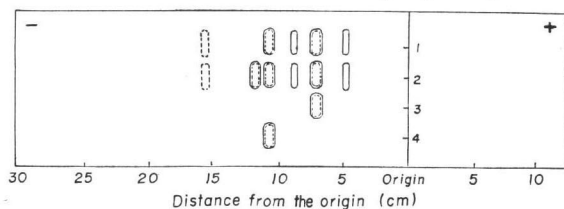


Table 1. Antibacterial spectra of F-1, 7-(5-amino-5-carboxypentamido)-3-methylthiomethyl-3-cephem-4-carboxylic acid (MTC) and cephalosporin C (CPC) by the agar dilution method

| Microorganism | MIC*, $\mu\text{g/ml}$ | | |
|---|------------------------|------|------|
| | F-1 | MTC | CPC |
| <i>Staphylococcus aureus</i> FDA 209 P | 40 | 40 | 20 |
| <i>Bacillus subtilis</i> PCI 219 | 10 | 10 | 5 |
| <i>Sarcina lutea</i> PCI 1001 | 40 | 40 | 20 |
| <i>Escherichia coli</i> IFO 3044 | 80 | 80 | 40 |
| <i>Proteus vulgaris</i> IFO 3045 | 20 | 20 | 5 |
| <i>Alcaligenes faecalis</i> ATCC 8750 | 5 | 5 | 5 |
| <i>Pseudomonas aeruginosa</i> IFO 3080 | 160 | 160 | 160 |
| <i>Comamonas terrigena</i> IFO 12685 | 0.25 | 0.25 | 0.25 |

* MIC: Minimal inhibitory concentration.

MIC was determined by incubating a nutrient agar containing an aliquot of sample to be tested for 18 hours at 28°C, on which the test organisms were streaked.

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