Identification of 7α-Hydroxycephalosporin C as an Intermediate in the Methoxylation of Cephalosporin C by a Cell Free Extract of *Streptomyces clavuligerus*

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Incubation of cephalosporin C with a cell free extract of *Streptomyces clavuligerus* resulted in the formation of 7α-hydroxycephalosporin C; the same crude enzyme preparation was shown to methylate synthetic 7α-hydroxycephalosporin C to form 7α-methoxycephalosporin C.

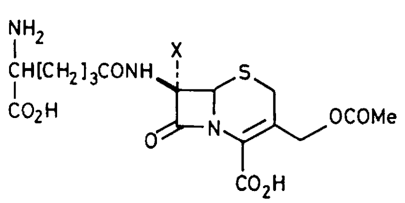
Streptomyces clavuligerus (ATCC 27064) produces the β-lactam antibiotic cephamycin C [7β-(5-amino-5-carboxyvaleramido)-3-carbamoyloxymethyl-7α-methoxy-3-cephem-4-carboxylic acid]. The methoxy group has been shown to be derived from molecular oxygen and methionine.[1,2] Furthermore, O'Sullivan and Abraham[3] using a cell free extract of *S. clavuligerus* demonstrated the introduction of a methoxy group into certain cephalosporins.

In this communication we present evidence for the methoxylation of cephalosporin C (**1**) by an extract of *S. clavuligerus* proceeding by a two-step reaction involving the formation of 7α-hydroxycephalosporin C (**2**) with subsequent methylation to yield 7α-methoxycephalosporin C (**3**).

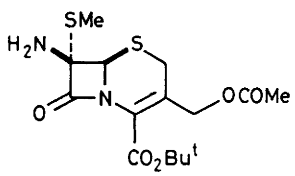
The preparation of a cell free extract and the reaction conditions for methoxylation of cephalosporin C were essentially as described by O'Sullivan and Abraham.[3] The reaction was stopped with acetic acid and the supernatant (1 ml) was applied to a QAE Sephadex A25 column (0.5 × 2 cm). The products were eluted with 0.2 M NaCl (1 ml) and submitted to h.p.l.c. Peaks (Figure 1) were detected at 9.0 min and 7.6 min corresponding to the retention times for synthetic 7α-methoxycephalosporin C (**3**)[4] and 7α-hydroxycephalosporin C (**2**), respectively. Neither peak was present when samples were treated with cephalosporinase (prepared from *Enterobacter cloacae* McDermott) prior to chromatography.

To confirm the identification of 7α-hydroxycephalosporin C (**2**) the oxygenation reaction was performed on a preparative scale from cephalosporin C (50 mg), using the described reaction conditions[3] but excluding *S*-adenosylmethionine. Purification on QAE Sephadex and HP20 columns yielded a white solid (2.6 mg) which was identical [by fast atom bombardment (f.a.b.) mass, n.m.r., and u.v. spectroscopy, and h.p.l.c.] to the synthetic hydroxycephem (**2**).

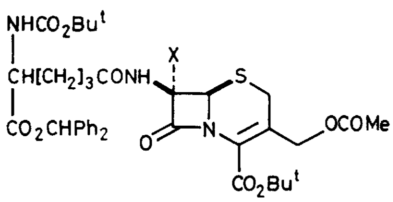
Methylation of synthetic 7α-hydroxycephalosporin C (**2**) was demonstrated using the same enzyme preparation and the reaction mixture contained 7α-hydroxycephalosporin C (**2**)



(1) X = H
(2) X = OH
(3) X = OMe



(4)



(5) X = SMe
(6) X = OH

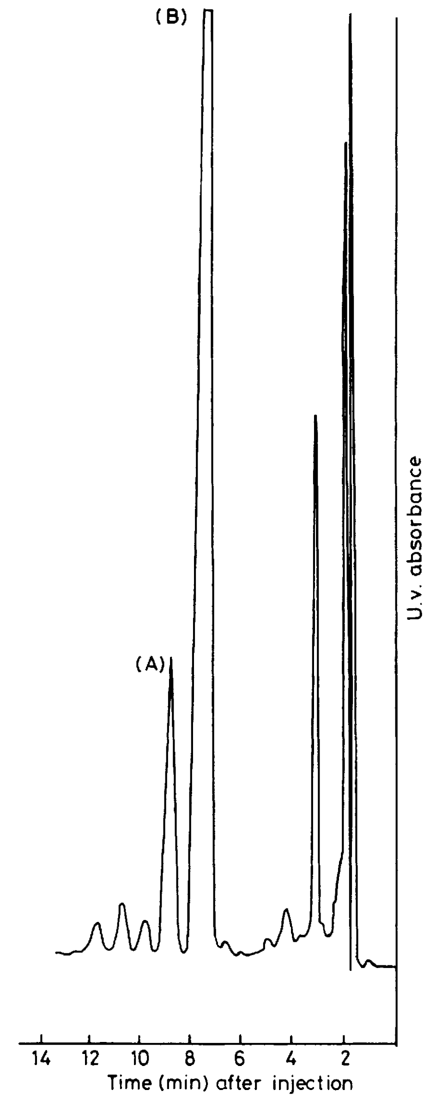


Figure 1. H.p.l.c. trace of the reaction products. H.p.l.c. conditions: C_{18} μ-Bondapak column eluted with 0.1 M NaH_2PO_4, pH 4.2 at 2 ml min^{-1}. U.v. monitor at 260 nm. (A) 7-Methoxycephalosporin C; (B) 7-hydroxycephalosporin C.

(1 mM), *S*-adenosylmethionine (1 mM) in MOPS [= 3-(*N*-morpholino)propanesulphonic acid] buffer (0.05 M, pH 7.5). 7α-Methoxycephalosporin C (**3**) was detected by h.p.l.c. (Figure 1).

7α-Hydroxycephalosporin C (**2**) was prepared as follows: the amine (**4**)[5] was acylated with the acid chloride derived from diprotected D-α-aminoadipic acid[6] and the resulting 7α-methylthiocephalosporin (**5**) was converted into the 7α-hydroxy derivative (**6**) by treatment with mercury(II) acetate in aqueous tetrahydrofuran. Deprotection of the ester (**6**) with trifluoroacetic acid–anisole and subsequent neutralisation afforded the potassium salt of 7α-hydroxycephalosporin C (**2**) {$[\alpha]_D^{20}$ + 102° (*c* 1 in H_2O); ν_{max} (KBr) 1760, 1660, and 1600 cm^{-1}; ^1H n.m.r. (D_2O) δ 1.7—1.9 (4H, overlapping m, $CHCH_2CH_2$), 2.08 (3H, s, CH_3CO_2), 2.42 (2H, t, *J* 7 Hz, CH_2CON), 3.29 and 3.65 (2H, ABq, *J* 17 Hz, 2-H), 3.73 (1H, t, *J* 7 Hz, C*H*NH), 4.65 and 4.81 (2H, ABq, *J* 13 Hz, CH_2OAc), and 5.10 (1H, s, 6-*H*); λ_{max} (H_2O) 260 nm (ε 7 200); positive ion f.a.b. mass spectrum, MH^+ 470, MK^+ 508}.

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References

1 J. O'Sullivan, R. T. Aplin, C. M. Stevens, and E. P. Abraham, *Biochem. J.*, 1979, **179**, 47.
2 J. G. Whitney, D. R. Brannon, J. A. Mabe, and K. J. Wicker, *Antimicrob. Agents Chemother.* 1972, **1**, 247.
3 J. O'Sullivan and E. P. Abraham, *Biochem. J.*, 1980, **186**, 613.
4 G. A. Koppel and R. A. Kochler, *J. Am. Chem. Soc.*, 1973, **95**, 2403.
5 T. Jen, J. Frazee, and J. R. E. Hoover, *J. Org. Chem.*, 1973, **38**, 2857.
6 S. Wolfe and M. G. Jokinen, *Can. J. Chem.*, 1979, **57**, 1388.