Conversion of 3-Exomethylene Cephalosporin C into Deacetyl Cephalosporin C in a Cell-free Extract from *Cephalosporium acremonium* (CW-19)

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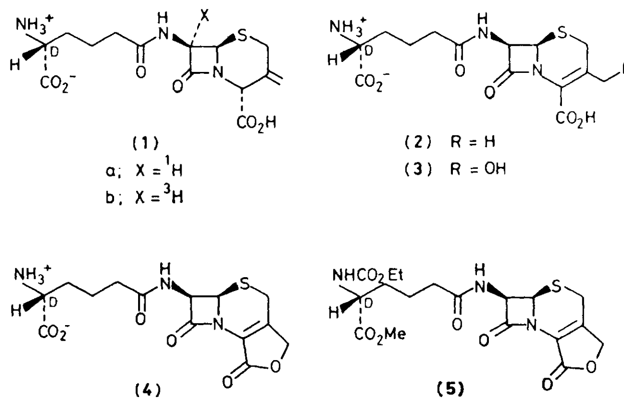
In a cell-free extract of the producing strain *C. acremonium* (CW-19) 3-exomethylene cephalosporin C was transformed into a strongly antibiotic substance, which was identified as deacetyl cephalosporin C.

During our studies on the conversion of penicillins into cephalosporins by cell-free extracts of the non-producing mutant of *C. acremonium* (M-0198) we observed that a biologically active β-lactam antibiotic was produced on incubation of 3-exomethylene cephalosporin C (**1**) with the enzyme extracts.[1]

We have now repeated these experiments with more active extracts from the producing strain *C. acremonium* (CW-19) and have identified this previously unknown metabolite. Thus when (**1a**) was incubated† with a cell-free extract from this organism, in the presence of α-oxoglutarate as a cofactor, there was obtained a strongly active cephalosporin (assayed against *E. coli* ESS).‡ This new product was identical to an authentic sample of deacetyl cephalosporin C (**3**) by electrophoresis (pH 3.5) followed by bioautography against *E. coli*, and also by its elution time on h.p.l.c. The boiled control showed no (**3**). When (**1b**)[1] was similarly incubated, h.p.l.c. showed a radioactive peak at the position expected of (**3**) and after treatment with formic acid, which converts (**3**) into lactone (**4**),[3] electrophoresis at pH 1.8 and 3.5 showed that the radioactive spot migrated with an authentic sample of (**4**).



† Methods of preparation of cell-free extracts, incubation, cofactors, analytical procedures *etc.*, have been described previously, *cf.* ref. 2.

‡ The precursor (**1**) has very little bioactivity.

Finally (**1a**) (2 mg) was incubated with the CW-19 extract in the presence of catalase§ and the resulting (**3**) was isolated by ion-exchange chromatography (Dowex-1, elution with water–pyridine–acetic acid, 94:3:3 by volume). After conversion, as before, into lactone (**4**) (0.46 mg, 23%) final purification by h.p.l.c. gave homogeneous lactone (**4**), identical to an authentic sample [^{1}H n.m.r. spectroscopy (300 MHz) and h.p.l.c.]. This sample was converted into the derivative (**5**)[4] (M^+ found 441.1206, $C_{18}H_{23}N_3O_8S$ requires 441.1206) which again was identical to an authentic sample [t.l.c. on silica gel, ^{1}H n.m.r. spectroscopy (300 MHz) and h.p.l.c.]. These extracts also contain the hydroxylating activity which converts (**2**) into (**3**)[5] and hence a possible explanation of our observations is that (**1**) was isomerised to (**2**) and subsequently hydroxylated to (**3**). However we were not able to detect radioactive (**2**) during bioconversions of (**1b**) into (**3**). Neither was any deuterium label found in (**4**) produced from [4-^{2}H]-3-exomethylene cephalosporin C[6] thereby eliminating any intramolecular 1,3-hydrogen shift in this postulated isomerisation of (**1**) to (**2**). Thus at this time the precise mechanism of conversion of (**1**) into (**3**) is unclear although perhaps it is most likely the result of hydroxylation of (**1**) by the activity which normally converts (**2**) into (**3**).[5]

§ Catalase protects the enzyme activity against deactivation by H_2O_2 and so improves the conversion yields.

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