On the Possible Role of the 3-Methylene Isomer of Deacetoxycephalosporin C in the Biosynthesis of Cephalosporins

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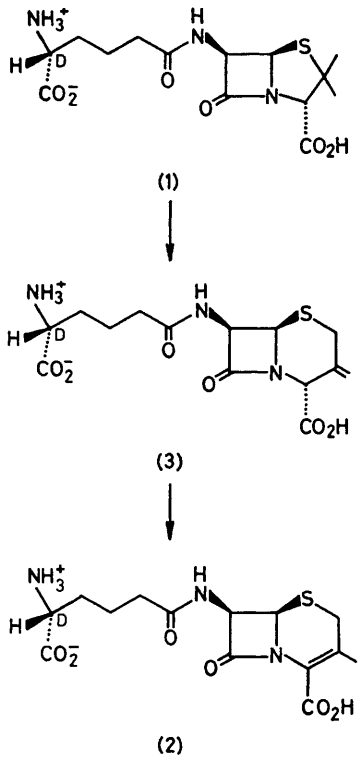
Summary The 3-methylene isomer of [7α-^{3}H]deacetoxycephalosporin C was incubated with a cell-free extract capable of converting penicillin N into deacetoxycephalosporin C; although there was no formation of deacetoxycephalosporin C from the 3-methylene isomer this compound was a powerful inhibitor of the penicillin N into cephalosporin bioconversion.

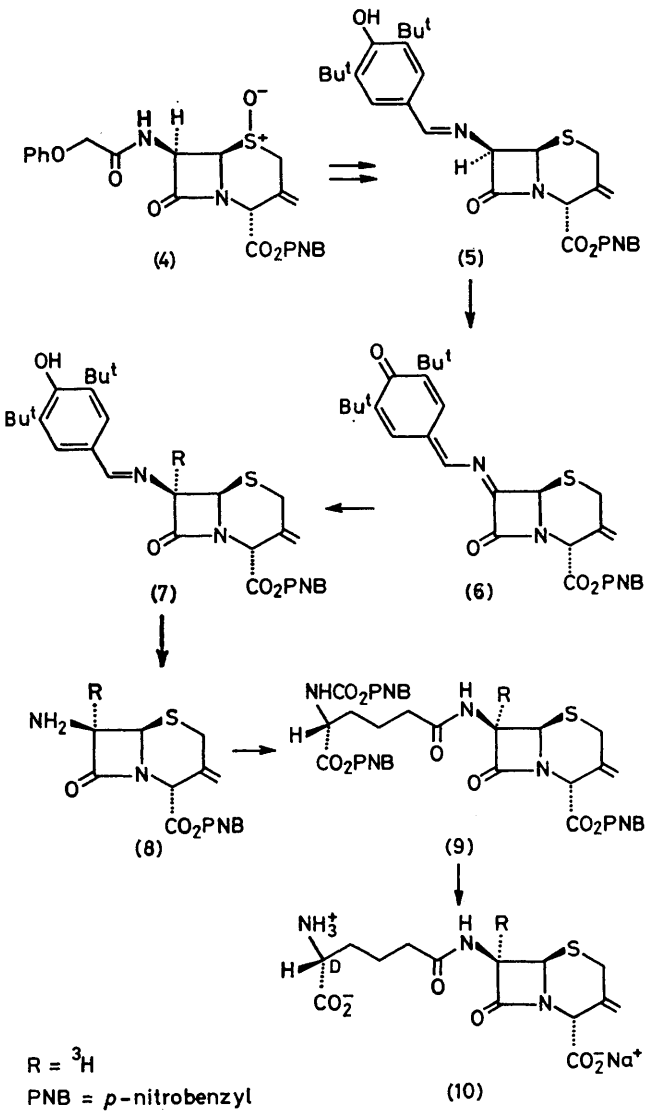
THE cell-free biosynthesis of deacetoxycephalosporin C (**2**) from singly and doubly labelled penicillin N (**1**) was established in an extract from *Cephalosporium acremonium* (M-0198).[1] Recently we suggested[2] the possible intermediacy of the 3-methylene isomer of deacetoxycephalosporin C (**3**)[3] in this process (Scheme 1). We have now tested this suggestion as follows. A sample of the [7α-^{3}H]-compound (**10**) was prepared from the sulphoxide (**4**)[4] *via* the Schiffs base (**5**) by oxidation (PbO_2) to (**6**), followed by reduction (NaB^3H_4) to (**7**) and hydrolysis (2,4-dinitrophenylhydrazine, toluene-*p*-sulphonic acid, EtOH) to the [7α-^{3}H]-amine (**8**) [21% from (**5**)].† This amine (**8**) was then coupled (53%,

† This sequence was previously shown to be stereospecific and was described in detail; *cf.* ref. 5.

2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline, CH_2Cl_2) with α-amino-α-(*p*-nitrobenzyl)-*N*-(*p*-nitrobenzyl-oxycarbonyl)-D-adipate[5] and the product (**9**) deprotected (H_2, 10% Pd/C, aq. tetrahydrofuran, $NaHCO_3$) to give (**10**) [92% from (**9**), specific activity 19·1 mCi/mmol] (Scheme 2).



SCHEME 1



SCHEME 2

A cell-free system, active in the conversion of penicillin N (**1**) into (**2**), was obtained by sonication of *C. acremonium* (M-0198). The labelled 3-methylene compound (**10**) [20 μg/ml; inactive against *Escherichia coli* 21/30 at this concentration] was incubated with the active extract.‡ Initial analysis of the incubation products by h.p.l.c., followed by t.l.c. on cellulose, gave chromatograms which were developed by fluorography and ninhydrin spray using authentic deacetoxycephalosporin C as a marker.‡ The results showed there was no conversion, by this extract, of (**10**) into labelled (**2**). Since such a conversion, if it occurred, could imply incorporation of protons from the medium into the product (**2**), we repeated the incubation but with unlabelled penicillin N in the presence of tritiated water (40 mCi/ml). Analysis of the so-formed deacetoxycephalosporin C (isolated by electrophoresis, pH 3·5) showed less than 20 atom % was incorporated. Taken together these results do not support the intermediacy of the 3-methylene isomer of deacetoxycephalosporin C (**3**) in cephalosporin biosynthesis. Further support for this conclusion comes from the observation that compound (**3**) is actually a powerful *inhibitor* of the penicillin N into cephalosporin bioconversion. Thus, in two experiments the unlabelled 3-methylene compound (**3**) [20 and 50 μg/ml, respectively] was added to an incubation mixture containing penicillin N (20 μg/ml).§ The formation of (**2**) in each case was strongly

‡ Methods of cell extract preparation, incubation, cofactors, *etc.*, and analytical procedures, including h.p.l.c., electrophoresis, and fluorography, have been described; *cf.* ref. 1.

§ The extract used in this experiment came from *C. acremonium* (CW-19), which is more easily prepared than that from *C. acremonium* (M-0198).

inhibited (87 and 94% inhibition, respectively, *vs.* the control).

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[4] We thank Eli Lilly and Company for a supply of (**4**); *cf.* S. Kukolja in 'Recent Advances in the Chemistry of β-Lactam Antibiotics,' ed. J. Elks, Special Publication No. 28, The Chemical Society, 1977, p. 181.
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