The Incorporation of DL-[4-$^2H_2$,5-^{13}C] Ornithine into Clavulanic Acid and *N*-Acetylglycylclavaminic Acid

Barrie W. Bycroft,[a] Audrey Penrose,[a] Janet Gillett,[b] and Stephen W. Elson*[b]

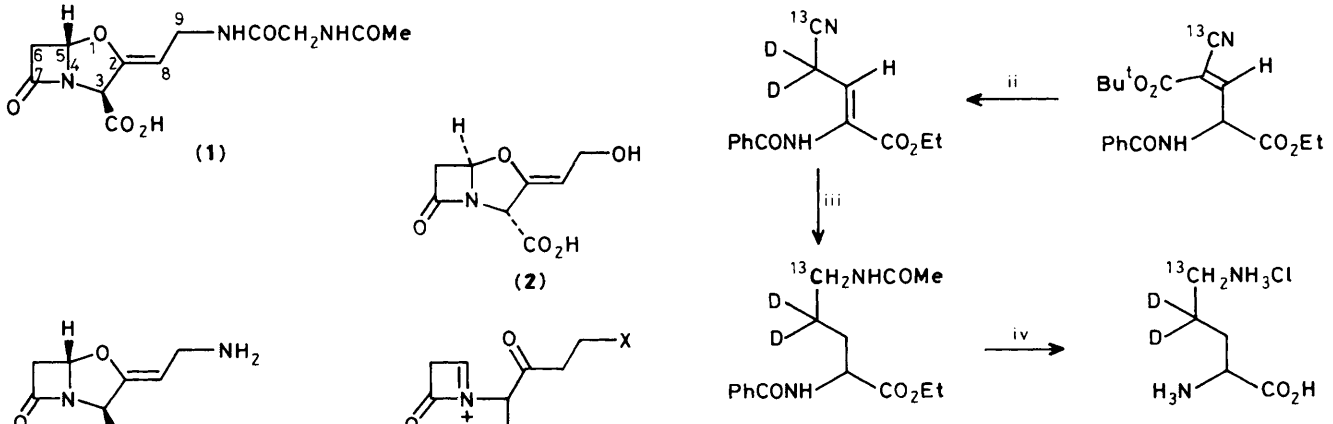
[a] *Department of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, U.K.*
[b] *Beecham Pharmaceuticals Research Division, Brockham Park, Betchworth, Surrey RH3 7AJ, U.K.*

Both the carbon and deuterium label of DL-[4-$^2H_2$,5-^{13}C] ornithine were incorporated into the novel β-lactam metabolite *N*-acetylglycylclavaminic acid, whereas only the carbon label was incorporated into clavulanic acid.

In the previous communication[1] we described the production of (3*S*,5*S*)-*N*-acetylglycylclavaminic acid (**1**), by a clavulanic acid (**2**) negative mutant of *Streptomyces clavuligerus*. The accumulation of (**1**) by this strain was cited as further evidence that clavaminic acid (**3**)[2] is a biosynthetic precursor of clavulanic acid. It was reasoned that in the mutant strain the pathway is blocked between (**3**) and (**2**), leading to (**1**) by acylation of the accumulated (**3**).

Ornithine is known[3] to be a well incorporated precursor of clavulanic acid and both (**1**) and (**3**) contain an intact ornithine residue. Furthermore, the incorporation of prochirally labelled [5-^{14}C, 5-^3H] ornithine[4] into (**2**) results in the specific loss of the *pro-S* hydrogen at C-5 and inversion of stereochemistry of the retained *pro-R* hydrogen. It occurred to us that these events could be associated not only with the change of the terminal amino constituent into a hydroxy group but also with the intriguing enantiomeric conversion of clavaminic acid (**3**) into clavulanic acid (**2**). To further investigate this important aspect DL-[4-$^2H_2$,5-^{13}C] ornithine was synthesised, as shown in Scheme 1. Its incorporation into (**2**) and (**3**) was investigated by feeding samples of this material into fermentations of a clavulanic acid producing strain of *S. clavuligerus* and also the blocked mutant. The resulting clavulanic acid (**2**) and *N*-acetylglycylclavaminic acid (**1**) were isolated as their benzyl and *p*-bromobenzyl esters respectively. Examination of the ^{13}C n.m.r. spectrum of the benzyl clavulanate resulting from the above labelling experiment revealed a substantial enhancement of the signal corresponding to C-9, indicating a ^{13}C-enrichment of 10.6 atom % above natural abundance. No other carbon centre was significantly enriched. No detectable up-field shift of the C-9 signal (which would be expected if deuterium were present at C-8) was observed.

In the case of the *p*-bromobenzyl *N*-acetylglycylclavaminate sample a small enhancement of the C-9 signal was also observed. However, in addition, a clearly observable



Scheme 1. D = deuterium, Tos = *p*-toluene sulphonyl. *Reagents and conditions*: i, NaH; ii, CF_3CO_2D, D_2O; iii, H_2, Pt_2O, acetic anhydride; iv, HCl, reflux.

(**1**)

(**2**)

(**3**)

(**4**)

β-deuterium up-field shift of 0.082 p.p.m. for this signal was apparent which was consistent with approximately 70% of the molecules bearing ^{13}C at C-9 also bearing deuterium at C-8. The total ^{13}C-enrichment at C-9 was calculated to be 3.0 atm % above natural abundance.

From the above results we conclude that the C_5-moiety of *N*-acetylglycylclavaminic acid and clavulanic acid are both derived from ornithine, the latter result being in agreement with previous observations using radioisotopes.[3] The results are consistent with clavaminic acid being a precursor of clavulanic acid rather than both metabolites being formed in parallel pathways by mechanistically identical cyclisation processes. The observed loss of the C-4 hydrogen of ornithine following incorporation into clavulanic acid as opposed to the retention in clavaminic acid suggests that a β-keto intermediate of the type (**4**) may be involved during the conversion of (**3**) to (**2**). A similar type of intermediate has been invoked to explain the base catalysed racemisation of clavulanates.[5]

We thank Mrs. Bridget Bawn (née Miller) and also our colleagues in the Physical and Analytical Department, Brockham Park, for expert assistance.

Received, 9th March 1988; Com. 8/00949J

References

1 S. W. Elson, J. Gillett, N. H. Nicholson, and J. W. Tyler, *J. Chem. Soc., Chem. Commun.*, preceding Communication.
2 S. W. Elson, K. H. Baggaley, J. Gillett, S. Holland, N. H. Nicholson, J. T. Sime, and S. R. Woroniecki, *J. Chem. Soc., Chem. Commun.*, 1987, 1739.
3 C. A. Townsend and M.-F. Ho, *J. Am. Chem. Soc.*, 1985, **107**, 1065.
4 C. A. Townsend, M.-F. Ho, and S.-S. Mao, *J. Chem. Soc., Chem. Commun.*, 1986, 638.
5 For discussion, see papers by C. E. Newell and by P. H. Bentley *et al.*, in 'Recent Advances in the Chemistry of β-Lactam Antibiotics,' ed. G. I. Gregory, Special Publication No. 38, Royal Society of Chemistry, London, 1980, pp. 151 and 175, respectively.