The Stereochemical Fate of (2*RS*,5*R*)- and (2*RS*,5*S*)-[5-^{3}H]Ornithine in Clavulanic Acid Biosynthesis

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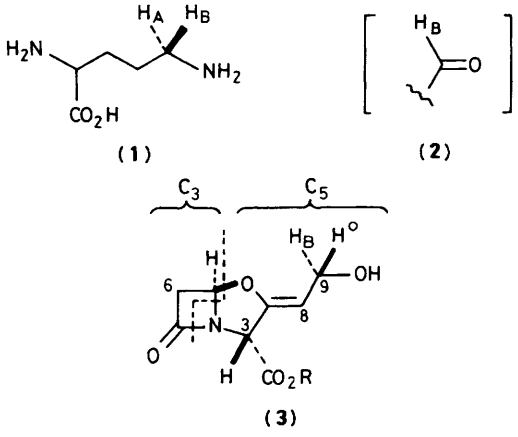
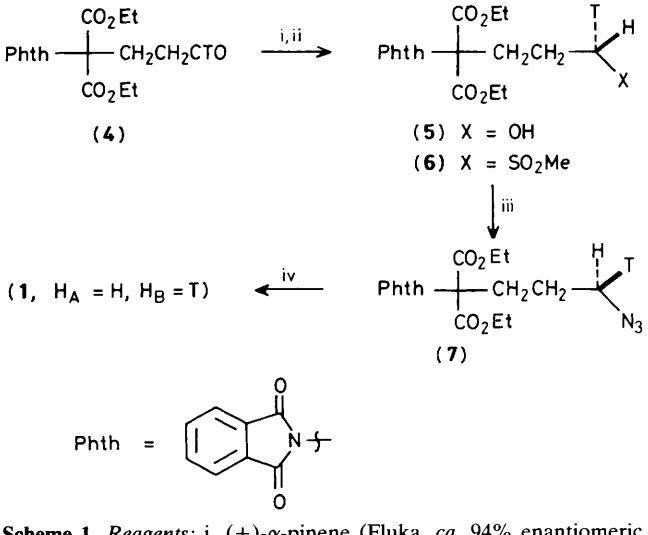
The 5-*pro*(*R*) hydrogen of ornithine is retained in the biosynthesis of clavulanic acid where it assumes the 9-*pro*(*S*) position with overall inversion of configuration.

Against expectation recent work has indicated a direct precursor role for ornithine in the biosynthesis of the C_5-segment of clavulanic acid (**3**, R = H).[1] Additional experiments have demonstrated that one half of the tritium label from (2*RS*,5*RS*)-[5-^{3}H,U-^{14}C]ornithine is lost on incorporation into (**3**) suggesting intermediate transamination or amine oxidation to an aldehyde as (**2**), which on eventual reduction would lead to the C-9 hydroxymethylene group of (**3**). The overall stereochemical course of these events has been examined with labelled specimens of ornithine (**1**).



(**1**) (**2**) (**3**)



(**4**) (**5**) X = OH (**6**) X = SO_2Me (**7**) (**1**, H_A = H, H_B = T)

Scheme 1. *Reagents:* i, (+)-α-pinene (Fluka, *ca.* 94% enantiomeric excess), 9-BBN, tetrahydrofuran (THF), room temp., 5 h, 71%; ii, $MeSO_2Cl$, Et_3N, CH_2Cl_2, room temp., 2 h, 93%; iii, NaN_3, acetone–H_2O (2:1), reflux, 18 h, 83%; iv, HOAc–conc. HCl (1:1), reflux, 8 h.

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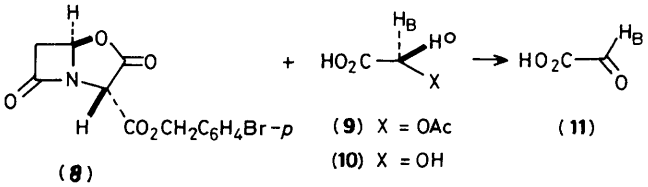
The preparation of (2*RS*,5*R*)-[5-^{3}H]ornithine (**1**, H_A = H, H_B = T) is illustrated in Scheme 1. The tritiated aldehyde (**4**) was obtained by reaction of diethyl phthalimidomalonate with acrolein[2] and reduction with sodium borotritiide followed by oxidation with buffered pyridinium chlorochromate.[3] Reaction of (**4**) in the presence of Midland's reagent,[4] (+)-α-pinene–9-borabicyclo[3.3.1]nonane (9-BBN), afforded ethyl (5*S*)-[5-^{3}H]-2-ethoxycarbonyl-2-phthalimido-5-hydroxy valerate (**5**),[5] which was in turn converted into (2*RS*,5*R*)-[5-^{3}H]ornithine (**1**, H_A = H, H_B = T) using standard procedures.‡ The corresponding (5*S*)-material was prepared by the same sequence of reactions but using (−)-α-pinene–9-BBN. D,L-Ornithine labelled stereorandomly at C-5 was obtained similarly by sodium borotritiide reduction of the adduct of diethyl phthalimidomalonate and acrolein.

The labelled ornithines were separately combined with D,L-[1-^{14}C]ornithine as internal standard and administered (0.5 mmol per 1.5 l) as before[1] to growing cultures of *Streptomyces clavuligerus* (ATCC 27064) in a glycerol-based fermentation medium.[6] The clavulanic acid produced was isolated as its crystalline *p*-bromobenzyl ester (**3**, R = *p*-$BrC_6H_4CH_2$). The incorporation data obtained are summarized in Table 1. Ornithine labelled stereorandomly at C-5 lost approximately one half of its tritium on incorporation into (**3**), while the stereoselectively labelled samples of (**1**) gave complementary results: the 5-*pro*(*R*) label (H_B) was largely retained while the 5-*pro*(*S*) label (H_A) was principally lost.§

Table 1. Incorporation of doubly labelled ornithines into *p*-bromobenzylclavulanate (**3**, R = *p*-$BrC_6H_4CH_2$).

		(**3**, R = *p*-$BrC_6H_4CH_2$) % ^{14}C Spec.	% ^{3}H
Substrate	^{3}H/^{14}C	Incorp.	Retained
(2*RS*,5*RS*)-[5-^{3}H,U-^{14}C]Orn.	9.55[a]	3.69	47.4
(2*RS*,5*R*)-[5-^{3}H,1-^{14}C]Orn.	6.41[a]	3.50	91.9
(2*RS*,5*S*)-[5-^{3}H,1-^{14}C]Orn.	7.26[a]	3.90	8.6

[a] Determined as the monohydrochloride.



(**8**) (**9**) X = OAc (**10**) X = OH (**11**)

‡ As noted in Scheme 1, step iv, conventional hydrolysis to generate the α-amino acid from the protected aminomalonate on heating overnight resulted in reduction of the azide as well. The mechanism of this reaction is not entirely clear but similar reductions of aryl azides have been noted in hot HBr–glacial acetic acid (P. A. S. Smith and B. B. Brown, *J. Am. Chem. Soc.*, 1951, **73**, 2438).

§ Taking into account the optical purities of the (+)- and (−)-α-pinenes used, *ca.* 94 and 81% respectively, it can be shown that the stereospecificity of the enzymic removal of the ornithine 5-*pro*(*S*) hydrogen is >90%.

To establish the final stereochemical orientation of H_B, the ornithine H-5 label retained in clavulanic acid, a further sample of (**3**, R = *p*-$BrC_6H_4CH_2$) was obtained having only tritium as tracer from incorporation of (2*RS*,5*RS*)-[5-^{3}H]ornithine. This material was converted into its 9-acetoxy derivative[7] and ozonolysed[8,9] to give the sensitive lactone (**8**) and acetylglycolic acid (**9**). The mixture was saponified; the tritiated glycolic acid (**10**) so obtained was purified by ion exchange chromatography and mixed with potassium [2-^{14}C]glycolate, and a portion converted into its *p*-bromophenacyl ester for accurate radiochemical determination. The remainder was assayed[10] for the orientation of tritium label with glycolate oxidase, an enzyme known[11] to remove stereospecifically the *pro*(*R*) hydrogen, H°, in the formation of glyoxylate (**11**). The latter, converted into its semicarbazone, retained 100% of the tritium present in (**10**). The enantiotopically labelled glycolate being inaccessible in this experimental context, the assay procedures were verified with a sample of racemic [2-^{3}H]glycolate prepared by the method of Stubbe and Abeles.[12] Analysis as above accordingly gave glyoxylate (**11**) possessing 47% of the tritium originally in (**10**).

In conclusion, removal of the 5-*pro*(*S*) hydrogen of ornithine (**1**, H_A) and subsequent delivery of a hydrogen (**3**, H°) to the *re*-face of a hypothetical aldehyde intermediate as (**2**) (as opposed to a sequence involving displacement or elimination–addition) leads to net stereochemical inversion at C-9 in clavulanic acid. It is noteworthy that the abstraction of the 5-*pro*(*S*) hydrogen from ornithine parallels the stereochemical behaviour of a series of bacterial and mammalian ω-amino transferases using α-oxoglutarate as acceptor[13] as well as that of diamine oxidase.[14] Moreover, the amine-to-alcohol conversion, or its reverse, accompanied by overall stereochemical inversion may be noted to have several analogies in natural product biosynthesis.[15]

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