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# Biosynthetic Precursors of Valclavam

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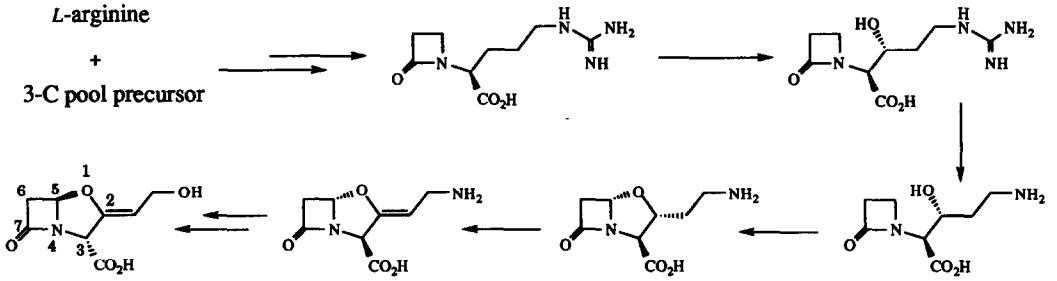
***Abstract***: The biosynthesis of valclavam was investigated by feeding radiolabelled potential precursors to fermentations of *Streptomyces antibioticus* ssp *antibioticus* Tü 1718. The preliminary results indicate that the primary metabolic precursors of valclavam are *L*-valine, *L*-arginine, *L*-methionine and a 3-C pool metabolite and suggest a common biosynthetic origin for clavulanic acid and valclavam.

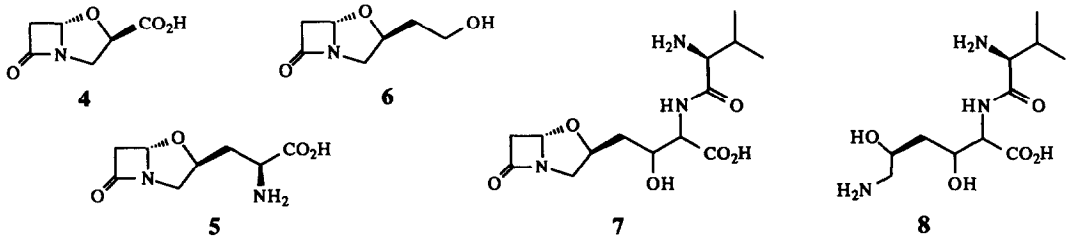
The important β-lactamase inhibitor clavulanic acid (**1**) is a member of the clavam group of secondary metabolites which are produced by *Streptomyces* spp. Clavulanic acid (**1**) and several simple derivatives are unique amongst the clavam family in possessing the 5*R*-stereochemistry. Biosynthetic investigations have, however, partially revealed an intriguing pathway to (**1**), in which two (and possibly more) intermediates (**2**,**3**) possess the antipodal stereochemistry at both C-3 and C-5 to clavulanic acid (**1**) itself, necessitating a subsequent double epimerisation process (Figure 1).[1]




**1** **3** **2** **9**

**Figure 1** Biosynthesis of clavulanic acid

*S. clavuligerus* produces several other clavams in addition to clavulanic acid (**1**), including clavam-2-carboxylate (**4**)[2] and clavalanine (**5**).[3] *S. antibioticus* produces 2-(2-hydroxyethyl)clavam (**6**) and valclavam (**7**),[4,5] whilst the clavamycins are produced by *S. hygroscopicus*.[6]



**4** **6** **5** **7** **8**

In order to investigate the possibility of a common biosynthetic origin for all clavams we[7] and others[8,9] have initiated studies on the biosynthesis of clavams other than clavulanic acid (**1**). This work has resulted in the revision of the structures of valclavam (**7**) and of Tü 1718B.[10] The latter was shown to be a degradation product of valclavam (**7**) and to have the structure **8**.[7] The incorporation of labelled proclavaminic acid (**9**) into clavam-2-carboxylate (**4**) has been reported by Townsend *et al.*,[8] indicating a common biosynthetic origin with clavulanic acid (**1**). Recently, [2,3-$^{13}C_2$]-*D,L*-proclavaminic acid (**9**) and [1-$^{13}$C]-*L*-valine were shown to be incorporated into valclavam (**7**),[9] an observation consistent with the theory of a shared pathway for all clavams (Figure 2). We report herein and in the following letter results that elaborate and lend further support to this theory.

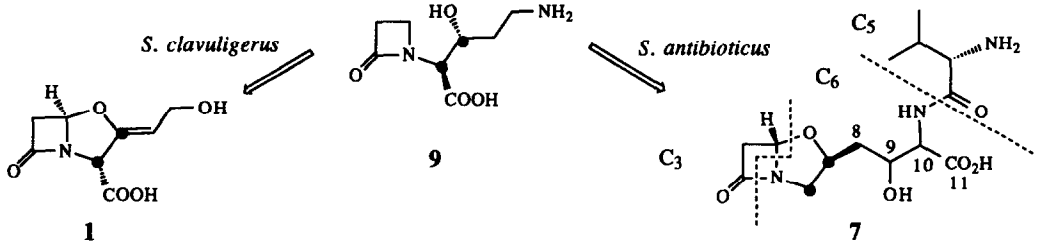



**Figure 2** Incorporation of proclavaminic acid (**9**) into valclavam (**7**) and clavulanic acid (**1**)

Fermentations of *S. antibioticus* ssp. *antibioticus* Tü 1718 for valclavam (**7**) production were carried out as 100-ml cultures in 500-ml Erlenmeyer flasks.[11,12] Feeding of labelled compounds was routinely performed between the 60th and 72nd hour after inoculation, whilst the culture filtrate was routinely collected between the 5th and 6th day. Two different fermentation media were used for the feeding experiments - one of which was the same as to that described by Rabenhorst[12] and the other included glycerol which was found to enhance valclavam production.[11] It has been observed,[13] during biosynthetic studies on clavulanic acid (**1**), that a glycerol-based medium can dramatically decrease the amount of $C_3$ precursor incorporated into clavulanic acid (**1**). Thus, glycerol was omitted in feeding experiments designed to determine the origin of the β-lactam carbons of valclavam (**7**). Isolation of valclavam (**7**) and its degradation fragment Tü 1718B (**8**) was performed as described previously.[7] Since Tü 1718B (**8**) is more stable than valclavam (**7**), it was the preferred choice for isolation and subsequent analysis for isotope incorporation. In cases where incorporation into Tü 1718B (**8**) was observed, the labelled positions could be further localised to the $C_6$ or $C_5$ unit of valclavam (**7**) (see Figure 2), since the $C_3$ unit is lost in the fragmentation process. In cases where the $C_3$ unit was a possible site of radiolabel incorporation, valclavam (**7**) was isolated intact. The results of these experiments are summarised in Table 1.

Assuming competent cellular uptake of common amino acids, it is concluded that *L*-lysine, glycine, *L*-glutamate and *L*-aspartate are not direct primary metabolic precursors for valclavam (**7**) biosynthesis. The significant level (6.8%) of incorporation of *L*-valine into Tü 1718B (**8**) suggests its direct utilisation as a precursor of valclavam (**7**). This is not surprising in view of the intact *L*-valyl residue in the $C_5$ unit. Indeed, [1-$^{13}$C]-*L*-valine had been shown earlier to be incorporated into valclavam (**7**), with enrichment of the $^{13}$C signal appearing exclusively at the carbonyl group of the $C_5$ unit.[9]

By analogy to the biosynthesis of clavulanic acid (**1**), it was speculated that the β-lactam carbons of valclavam (**7**) could be derived from compounds in the $C_3$ pool which are interconvertable through primary metabolism. Incorporation of glycerol,[14,15] pyruvate,[16] *D*-glycerate,[16] *D*-lactate[17] and β-hydroxypropionate[18] into clavulanic acid (**1**) has been observed. The figures in Table 1 indicate a substantial level of incorporation of glycerol, *L*-lactate, *D*-lactate and pyruvate into valclavam (**7**), consistent with the β-lactam carbons of **7** being

derived from the $C_3$ pool. The results for glycerol, where both valclavam (**7**) and Tü 1718B (**8**) were analysed for radiolabel incorporation, unambiguously localise the site of labelling at the β-lactam ring.

| Compound fed[a] | No. of expt | Analysed as valclavam (**7**) or Tü 1718B (**8**) | Av. level of specific incorporation (%) |
|---|---|---|---|
| [U-$^{14}$C]-*L*-valine | 2 | 8 | 6.8 |
| [U-$^{14}$C]-*L*-ornithine | 2 | 8 | 2.9 |
| [U-$^{14}$C]-*L*-arginine[b] | 2 | 8 | 18.6 |
| [$^{14}$COOH]-*L*-arginine | 1 | 8 | 0.7 |
| [$^{14}CH_3$]-*L*-methionine | 2 | 8 | 5.8 |
| [U-$^{14}$C]-*L*-lysine | 2 | 8 | 0.8 |
| [U-$^{14}$C]-glycine | 2 | 8 | 0.9 |
| [U-$^{14}$C]-*L*-glutamate[b] | 1 | 8 | 0.6 |
| [U-$^{14}$C]-*L*-aspartate | 1 | 8 | 0.7 |
| [U-$^{14}$C]-glycerol | 2 | 7 | 5.4 |
| [U-$^{14}$C]-glycerol | 1 | 8 | 0.6 |
| [U-$^{14}$C]-*L*-lactate | 1 | 7 | 5.2 |
| [U-$^{14}$C]-*D*-lactate | 1 | 7 | 4.5 |
| [1-$^{14}$C]-pyruvate | 1 | 7 | 2.0 |

(a) All radiolabelled compounds were fed at 0.1 mmole per 100-ml fermentation at a specific activity of 100 μCi/mmole.
(b) The specific activity of Tü 1718B (**8**) was multiplied by five-quarters to take into account the loss of 1-$^{14}$COOH.

**Table 1** Summary of whole-cell feeding experiments with *S. antibioticus* ssp *antibioticus* Tü 1718

The failure to observe *L*-lysine incorporation implies that this amino acid is not a direct precursor for valclavam (**7**), although the $C_6$ unit is a masked β,δ-dihydroxylysyl residue. It was therefore interesting to observe the high incorporation of [U-$^{14}$C]-*L*-arginine into the Tü 1718B (**8**), pointing to its possible role as a precursor for the $C_6$ unit of valclavam (**7**). Moreover, the lack of radiolabel incorporation from [$^{14}$COOH]-*L*-arginine argues for a regiochemistry of incorporation that is the same as that observed in clavulanic acid (**1**) biosynthesis, as depicted in Figure 3. Thus C-2, C-3, C-8 and C-9 of the $C_6$ unit of valclavam (**7**) are postulated to originate from *L*-arginine. The lower level of incorporation of [U-$^{14}$C]-*L*-ornithine is consistent with the fact that it has to be converted, *via* the urea cycle, into *L*-arginine which is the point of entry into the clavulanic acid pathway.[17] There remains the question of the origin of C-10 and C-11 of the $C_6$ unit of valclavam (**7**). The low level of incorporation of glycine eliminates the possibility of it being a precursor for C-10 and C-11 of the $C_6$ unit. We were surprised to find a significant incorporation of [$^{14}CH_3$]-*L*-methionine into Tü 1718B (**8**), suggesting that at least one of the two carbons in the $C_6$ unit originate from the methyl groups of *S*-adenosylmethionine (see Figure 3). The unexpected origin of the partial carbon skeleton of β-lactam metabolites from 1-C pool precursors has precedent in the biosyntheses of carbapenem antibiotics[19] and tabtoxin[20] where, respectively, the hydroxyethyl side chain and the β-lactam carbonyl carbon are derived from the 1-C pool. The mechanism by which secondary metabolites are apparently elaborated by addition of carbons from the 1-C pool is an intriguing one and will undoubtedly be the subject of further study.

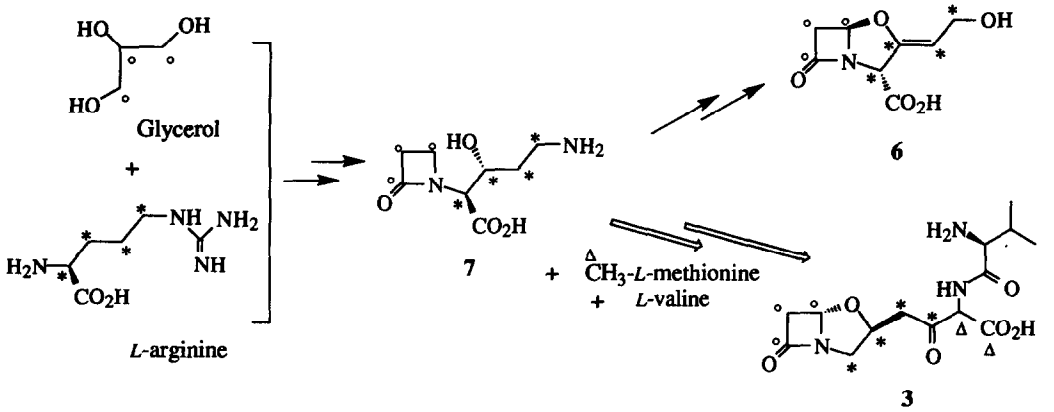



**Figure 3** Postulated biosynthetic pathway to valclavam (**7**), compared with that for clavulanic acid (**1**)

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