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Revised Structures for Tü 1718B and Valclavam

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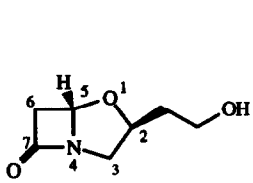
Abstract: The structures of Tü 1718B and valclavam, antibiotics produced by *Streptomyces antibioticus ssp. antibioticus* Tü 1718, have been reassigned as valyl-β,δ-dihydroxylysine (**4**) and the clavam (**5**) respectively, on the basis of NMR experiments and the observation that the former is a degradation product of the latter.

Two β-lactam antibiotics have been isolated from the culture broths of *Streptomyces antibioticus ssp. antibioticus* Tü 1718. The first metabolite,[1] Tü 1718A, was assigned the structure of (2S,5S)-2-(2-hydroxyethyl)clavam (**1**), a proposal which was subsequently confirmed by synthesis.[2] The second metabolite,[3,4] Tü 1718Z, or valclavam, was provisionally assigned the structure **2**. Unlike most other naturally occurring β-lactams possessing antibacterial activity, these clavams do not inhibit peptidoglycan biosynthesis. Instead, they effect bacteriostatic action through the inhibition of methionine biosynthesis in chemically defined media.[5] In addition to the clavams, a dipeptide antibiotic,[6] Tü 1718B, was isolated from the same organism. It was assigned the provisional structure **3**, which was recently disproved by synthesis of the two possible epimers.[7]



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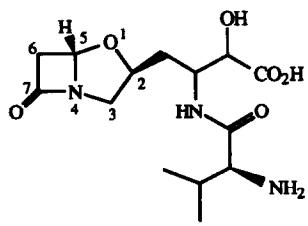

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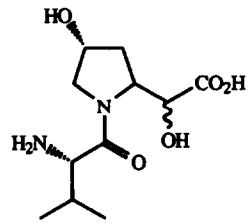

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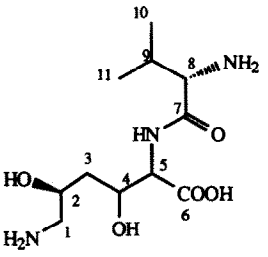
We have initiated biosynthetic studies on valclavam and these studies have led us to isolate a degradation fragment , which we have provisionally assigned as structure **4** on the basis of analytical studies. The NMR and mass spectrometric data for **4** were found to be analogous to those reported for Tü 1718B,[6] leading to the provisional reassignments of the structure of Tü 1718B as **4** and valclavam as **5** (The configurations at C-2 of **4** and C-2 and C-5 of **5** as drawn are tentatively inferred from those of the defined C-2 and C-5 configurations of **1**, based on the similar biological activities[5] of **1** and **5** and their possible biogenetic relationship).

Thus, valclavam was isolated by direct HPLC of the broth filtrate, utilising a methanol-phosphate gradient. The 500 MHz ^{1}H NMR spectrum of a freshly prepared sample was almost identical to that accrued during the original structural elucidation at Ciba-Geigy.[8] Upon standing in aqueous solution, valclavam was found to degrade and subsequent HPLC in ammonium bicarbonate buffer led to the isolation of a stable fragment.



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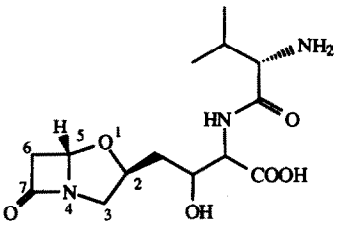
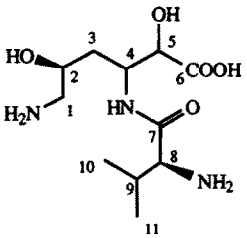


5

The fragment was found to be active against *Escherichia coli* ATCC 12435, but in contrast to valclavam its activity was not reversed by methionine, indicating an alternative mode of action. The UV absorption spectrum of the fragment showed a maximum at 210 nm, whilst the circular dichroism spectrum showed a negative Cotton effect at 217 nm and a positive band below 200 nm.[9] The electrospray mass spectrum gave $MH^+ = 278$ {100%} and $[M-H_2O]H^+ = 260$ {16%}. A 500 MHz ^1H-^1H COSY spectrum was consistent with the connectivity as indicated in structures **4** or **6**, the latter of which would be an anticipated degradation product of the previously proposed valclavam structure **2**. Comparison of the analytical data (biological activity, UV, CD, MS, ^1H NMR) for the fragment with those reported for Tü 1718B led us to conclude they were probably the same compound. Assignment of the fragment / Tü 1718B as structure **4** rather than **6** was based on the application of long-range ^1H-^{13}C correlation experiments and, in view of the small sample quantities available, "inverse" proton detected experiments in particular were used.



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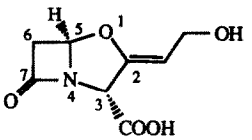

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Signals for all 11 carbon atoms of **4** could be observed after a 12-hr 1D acquisition. Assignment of all the protonated carbon resonances was achieved via a HMQC shift correlation experiment.[10] Long-range (two- and three-bond) ^1H-^{13}C correlations were obtained from HMBC experiments.[11] These were recorded on a basic sample (pD 7.5 - 8.5, from the residual ammonium bicarbonate) and on the same sample after acidification (pD < 1.0). This was necessary because at the basic pD many proton resonances were broadened, most notably 8-H, and failed to show correlations. These resonances were readily sharpened on acidification. Correlations were recognised directly from the 2D plot and from columns of the 2D spectrum corresponding to the ^{13}C dimension. Chemical shift assignments of **4** are given in Table 1, and observed long-range correlations summarised in Table 2. All long-range correlations were consistent with the dipeptide moieties identified in COSY spectra and were sufficient to assign the two carbonyl resonances unambiguously. The regiochemistry was established by the observation of long-range correlations of protons 4 and 5. Thus, only 5-H showed correlations with both carbonyl resonances, whereas 4-H correlated with C-6 only (Fig. 1). Such observations would be consistent with structure **4** only.

Figure 1 : Sections of ^{13}C columns of a HMBC experiment recorded for the basic sample of dipeptide **4**. Long-range correlations to carbonyl groups are shown for protons 4 and 5.

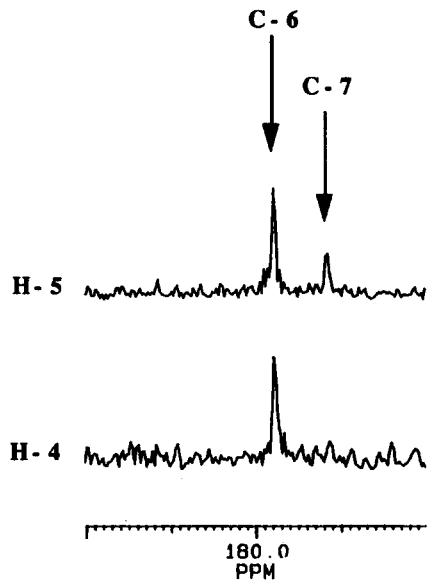

Table 1 : Proton and carbon assignments of dipeptide **4** at pD < 1.0 and *ca.* 8.0, and at 311 K. Shifts are relative to TSP at 0.00 ppm. Proton shifts were measured in a 1D spectrum whereas carbon shifts were taken from columns of the 2D HMBC experiment.

Carbon atom	Acidic pD		Basic pD	
	^{1}H	^{13}C	^{1}H	^{13}C
1	2.97, 3.18	47.3	2.99, 3.20	47.2
2	4.05	68.4	4.08	68.4
3	1.78	40.5	1.75	40.9
4	4.47	71.0	4.28	72.0
5	4.68	59.6	4.33	61.6
6	-	175.8	-	178.9
7	-	172.8	-	175.9
8	4.00	61.3	3.68	62.8
9	2.30	33.0	2.16	33.9
10	1.05	19.6	1.00	19.8
11	1.08	20.6	1.03	21.2

Thus, the structures of Tü 1718B and valclavam are now reassigned as valyl-β,δ-dihydroxylysine (**4**) and **5** respectively. Experiments are underway to clarify the remaining stereochemical questions and hence establish the complete structure of these compounds. By analogy with biosynthetic studies on clavulanic acid (**7**),[12,13] the structural reassignment of valclavam as **5** may suggest that the biogenetic precursors for the three- and six-carbon segments of valclavam are glycerol and lysine respectively. This hypothesis is currently under

investigation. An alternative possibility was proposed by Townsend[14] who has suggested that the initial stages of the pathways to **7** (in *S. clavuligerus*) and **1** (in *S. antibioticus*) are essentially identical.

Table 2 : Summary of the long-range ^{1}H-^{13}C correlations observed in HMBC experiments performed on **4**

Proton	Carbon-13 (Basic)	Carbon-13 (Acidic)
1[a]	2	2, 3
2[b]	-	-
3	1, 2, 4, 5	1, 2, 4, 5
4	2, 3, 6	3, 6
5	3, 4, 6, 7	6, 7
8[c]	-	7, 9, 10, 11
9	7, 8, 10, 11	10, 11
10	8, 9, 11	8, 9, 11
11	8, 9, 10	8, 9, 10

[a] Correlations were observed for the high-field proton only.
[b] No correlations were observed for proton 2, presumably because of the high multiplicity and low intensity of the proton resonance.
[c] In basic solution, no correlations were observed for proton 8 as the resonance was very broad. This resonance was a sharp doublet in acidic solution and gave rise to the listed correlations.

We are very grateful to Prof Dr Hans Zähner (University of Tübingen) for the generous gifts of (1) a slope of *S. antibioticus ssp. antibioticus* Tü 1718, (2) a sample of natural valclavam and (3) a photocopy of the Ph.D. dissertation by J. Rabenhorst. K.C.G. thanks the Singapore Economic Development Board for a scholarship.

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