The Edman Degradation of Vancomycin: Preparation of Vancomycin Hexapeptide

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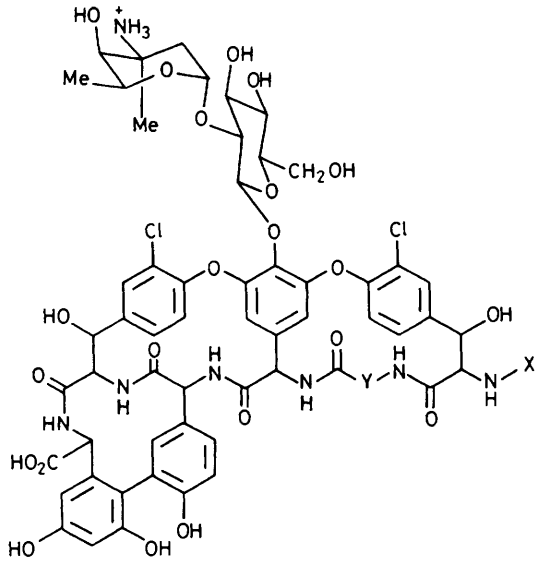
The preparation and characterisation of the hexapeptides derived from vancomycin and vancomycin CDP-1 by Edman degradation are described.

Vancomycin (**1**) is an antibiotic isolated from *Nocardia orientalis* (formerly called *Streptomyces orientalis*), which acts by inhibiting cell-wall biosynthesis in bacteria. It is clinically important in the treatment of methicillin-resistant *Staphylococcus aureus* infections and *Clostridium difficile*-induced pseudomembranous colitis.[1] Since bacteria do not appear to develop efficient resistance mechanisms to vancomycin, it (or other members of the vancomycin group) is likely to assume greater therapeutic significance in light of the increasing incidence of strains of bacteria resistant to other antibiotics.[2] Detailed studies on the mode of action of vancomycin have been reported previously.[3]

Although the synthetic modification of naturally derived antibiotics is a well precedented procedure,[4] at the present time no published protocol exists for the selective variation of any part of the vancomycin molecule, with the essentially trivial exceptions of the preparation of the aglycone,[5] and of mono- and di-dechlorovancomycin.[6] We describe below a procedure for the selective removal in high yield of the N-terminal residue of vancomycin by Edman degradation. This leaves a hexapeptide which should be amenable to systematic modification in the N-terminal region of the molecule. Since the binding of vancomycin to cell-wall mucopeptide precursors, such as Ac-D-Ala-D-Ala, constitutes a well characterised receptor–substrate system, these modifications have the potential to add greatly to our understanding of molecular recognition phenomena.

The Edman degradation is a well known and valuable procedure which allows the stepwise cleavage of the N-terminal residue from a peptide or protein molecule.[7] It has previously been applied to the vancomycin group of glycopeptide antibiotics as an aid to the structure determination of avoparcin.[8] In this case however only the thiohydantoin derivatives from the cleavage steps were characterised. We reasoned that the Edman procedure could also be applied to vancomycin and that isolation of the other cleavage product, which we name vancomycin hexapeptide, should also be possible.

Accordingly, reaction of vancomycin hydrochloride with 1.2 equiv. of phenyl isothiocyanate in pyridine/water afforded a single compound by reversed-phase h.p.l.c. analysis. A 250 MHz n.m.r. spectrum of this material showed a downfield shift of the *N*-methyl group of *N*-methyl-leucine by 0.75 p.p.m. Together with a molecular weight of 1582 determined by fast atom bombardment (f.a.b.) mass spectrometry, this indicates that selective reaction had occurred at the N-terminus of vancomycin to afford the mixed thiourea (**2**). Several conditions for the acid-catalysed cleavage of (**2**) were examined. The optimum conditions were found to be treatment with trifluoroacetic acid in dichloromethane. After separation of material soluble in ether this procedure afforded material which was shown by reversed-phase h.p.l.c. to be essentially homogeneous. F.a.b. mass-spectrometric analysis indicated a molecular weight of 1320, and this together with a complete



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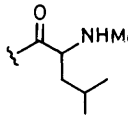
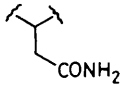
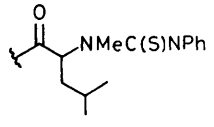
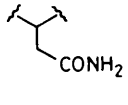
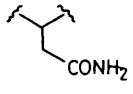
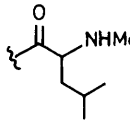
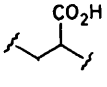
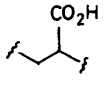
(1)		
(2)		
(3)	H	
(4)		
(5)	H	
(6)	Structure as in (3), less the disaccharide moiety	

assignment of the 400 MHz n.m.r. spectrum of the material in dimethyl sulphoxide (DMSO) solution indicated that the desired cleavage of the *N*-methyl-leucine had indeed occurred to afford (**3**).

An interesting feature of the n.m.r. spectrum of (**3**) is the change in chemical shift of the asparagine β-protons in going from native vancomycin (**1**) to the hexapeptide (**3**). In (**1**), in DMSO solution, these occur at δ 2.20 and 2.45 both with a time-averaged coupling constant of 7 Hz to the α-CH of the asparagine residue (designated *x*3 in our previously described nomenclature).[9] In the hexapeptide (**3**) these occur at δ 2.15 and 2.90 with coupling constants of <2 and 8 Hz to *x*3 respectively, indicating that there is a preferred conformer about the α–β bond of the asparagine residue. Furthermore, the chemical shifts of these β-protons in (**3**) are similar to those found in CDP-1 (**4**), a rearrangement product of vancomycin. In CDP-1 the asparagine is isomerised to an iso-aspartate, with introduction of the β-methylene into the peptide backbone.[10] This was particularly disturbing since CDP-1 lacks any antibiotic activity, and hence if such a rearrangement occurred this would thwart our stated intention of preparing new compounds and studying their antibiotic properties. CDP-1 is known to exist as an equilibrium mixture of two atropisomers in which ring 2 is slowly rotating with a half-life of several hours, allowing their separation by h.p.l.c.[11] No evidence for such isomerism was found for (**3**) by n.m.r. spectroscopy, nor could any separation be induced on detailed h.p.l.c. examination. Unequivocal proof that (**3**) had not undergone rearrangement was obtained by preparation of CDP-1 hexapeptide.

CDP-1, prepared as described by Marshall,[5] was treated under identical conditions to those used for vancomycin to give CDP-1 hexapeptide (**5**). This material was separable into a major and minor component by h.p.l.c. Comparison of n.m.r. data on these separated components with those of the original mixture indicated that equilibration did indeed occur over an extended time period. This was confirmed by h.p.l.c. analysis which showed that the separate isomers eventually gave rise to an identical equilibrium mixture when stored in solution. In the n.m.r. spectrum of the major CDP-1 isomer the iso-asparagine β-protons occurred at δ 1.95 and 3.00, and in the minor isomer at δ 2.10 and 2.97, clearly different from the shifts observed in (**3**). Neither of the CDP-1 isomers co-eluted with (**3**) on h.p.l.c. analysis, confirming that no rearrangement of the latter had taken place.

We have prepared aglucovancomycin hexapeptide (**6**) in similar fashion to (**3**), and further studies on this and (**3**) are currently being undertaken.

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