On the Biosynthesis of the Antibiotic Vancomycin

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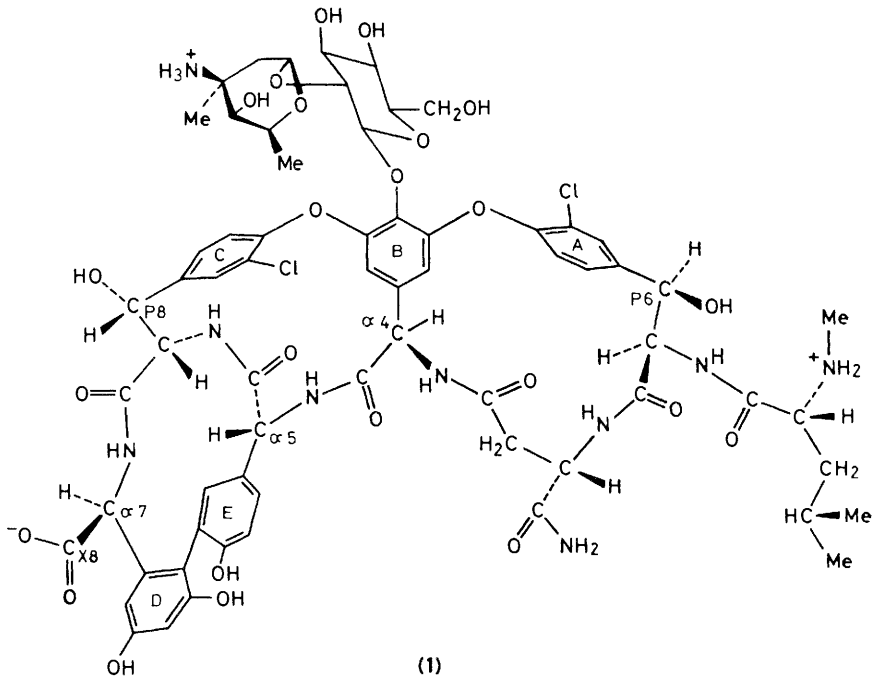
In the biosynthesis of vancomycin by *Streptomyces orientalis* it has been shown that di-*m*-hydroxyphenylglycine can be derived from acetic acid, whereas *p*-hydroxyphenylglycine and *m*-chloro-β-hydroxytyrosine are derived from tyrosine.

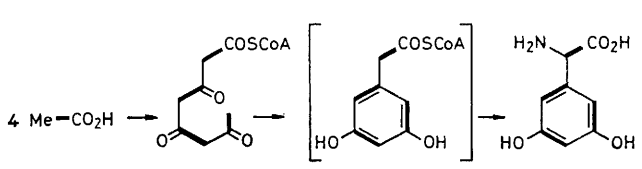
Vancomycin is a glycopeptide antibiotic which is isolated from *S. orientalis* and functions by interfering with bacterial cell wall synthesis.[1] Its structure was inferred from the *X*-ray analysis of a derivative[2] but a study of the molecule by ^{1}H n.O.e. difference spectroscopy has required a minor revision of the structure, in the orientation of ring A, to give (**1**).[3] The ^{13}C n.m.r. spectrum of vancomycin has recently been assigned[4] and this has allowed an investigation of the biosynthesis of the antibiotic aglycone using precursors labelled with ^{13}C and with both ^{13}C and ^{2}H. In particular, we have elucidated the origins of the unusual aromatic amino acid residues.

A fermentation of *S. orientalis* (300 ml) was supplemented with [1,2-$^{13}C_2$]acetate (99 atom %, 320 mg) in two equal portions 2 and 3 days after inoculation. Vancomycin was isolated from the culture after 5 days and examined by ^{13}C n.m.r. spectroscopy at 100 MHz. The $^{13}C\{^1H\}$ spectrum revealed enrichment with [1,2-$^{13}C_2$]acetate at each of the carbon atoms of ring D and at the adjacent two carbon atom unit α7–X8 [see (**1**)]. This pattern of labelling is consistent with the conclusion that the amino acid di-*m*-hydroxyphenylglycine can be assembled by the cyclisation of a tetraketide (Scheme 1).

The results of four other feeding experiments, with labelled



(1)

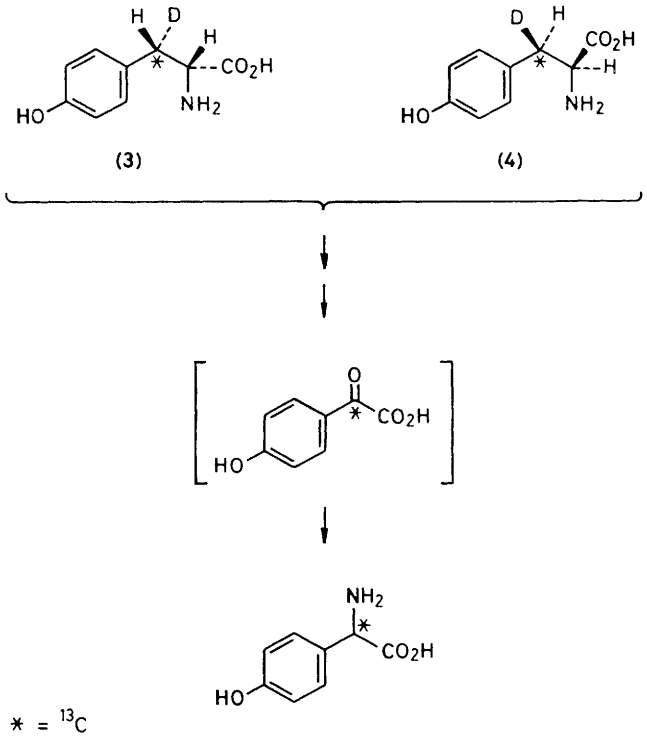


Scheme 1

Table 1. Incorporation of labelled amino acids into vancomycin (**1**).[a]

Substrate	Enhancement of ^{13}C signal[b] P6	P8	α4	α5
(2*RS*)-[2-^{13}C]-*p*-HPG	—	—	11.2	9.8
(2*R*,3*R*)-[2-^{2}H,3-^{2}H$_1$,3-^{13}C]tyrosine (**2**)	4.5	4.5	4.8	5.6
(2*S*,3*S*)-[2-^{2}H,3-^{2}H$_1$,3-^{13}C]tyrosine	1.2	1.2	1.7	1.9
(2*RS*,3*SR*)-[3-^{2}H$_1$,3-^{13}C]tyrosine [(**3**) + (**4**)]	2.9	3.5	5.0	5.8

[a] Typically the substrate was added two days after inoculation of the culture (300 ml) and the antibiotic was isolated two days later. [b] Enhancement is the factor by which the height of a signal in the $^{13}C\{^{1}H\}$ spectrum of the enriched compound is increased over the height at natural abundance.



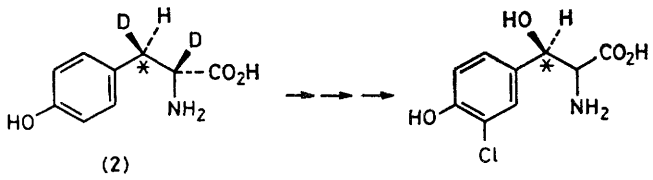
* = ^{13}C

Scheme 2

substrates which were specially prepared for this study, are recorded in Table 1.

Vancomycin contains two *p*-hydroxyphenylglycine (*p*-HPG) residues which both have the *R*-configuration (rings B and E). These were labelled at the positions α4 and α5 by incorporation of (±)-[2-^{13}C]-*p*-HPG (90.4 atom % ^{13}C). Furthermore, enrichment of the same signals was observed in vancomycin obtained from a culture which had been fed (2*R*,3*R*)-[2-^{2}H,3-^{2}H$_1$,3-^{13}C]tyrosine (**2**) (90.4 atom % ^{13}C, 98 atom % 3-^{2}H, 80% stereochemical purity at C-3). This result supports an earlier proposal[4] that the *p*-HPG units are biosynthesised from tyrosine.

Two more conclusions may be drawn from the feeding experiment with (**2**): (i) D-tyrosine can act as a precursor for the biosynthesis of *m*-chloro-β-hydroxytyrosine (CHT) and (ii) β-hydroxylation in the conversion of tyrosine to CHT occurs with retention of configuration at the β-carbon atom. These points are considered in turn. (i) In the experiment with (**2**), P8 is enhanced to the same level as P6 even though the configuration at the α-carbon atom of this CHT residue, ring C, is *S* (*i.e.*, opposite to that of the labelled substrate and the other CHT residue, ring A). Inversion at this centre must occur at some stage in the biosynthesis. It seems most likely that D-tyrosine is incorporated to provide ring C following



Scheme 3

initial α-epimerisation since the normal *in vivo* pathway is expected to employ the endogenous L-amino acid.

Further evidence for the effective incorporation of D-tyrosine came from the vancomycin which was enriched with (2*RS*,3*SR*)-[3-$^{2}H_{1}$,3-^{13}C]tyrosine [(**3**) + (**4**)] (90.4 atom % ^{13}C, 97 atom % ^{2}H, 80% stereochemical purity at C-3). The low level of enhancement of the signals for P6 and P8, relative to those for α4 and α5, suggests the presence of some ^{2}H at these two positions. The 100 MHz ^{13}C{^{1}H, ^{2}H} n.m.r. spectrum of this sample showed two intense, sharp singlets 0.53 and 0.46 p.p.m. upfield of the normal protonated P6 and P8 signals, respectively. These singlets are assigned to P6 and P8, shifted by the attachment of ^{2}H. Isotopic shifts of this magnitude are well documented.[5] The partial retention of ^{2}H suggests that L- and D-tyrosine are both incorporated from the racemic mixture. (ii) In the ^{13}C{^{1}H} spectrum of vancomycin enriched with (**2**) the signals for P6 and P8 were enhanced almost as much as those for α4 and α5, which are carbon atoms that do not retain any of the ^{2}H present in the substrate. [Loss of ^{2}H at α4 and α5 was inferred from the ^{13}C{^{1}H,^{2}H} spectrum described above which lacked any isotopically-shifted signals for these two carbon atoms (see Scheme 2).] Therefore, a low proportion of ^{2}H is present at P6 and P8. These two carbon atoms both have the *R*-configuration in vancomycin and so the loss of ^{2}H at C-3 from (**2**) demands that hydroxylation has gone with retention of configuration (Scheme 3).

The feeding experiment using the enantiomer of (**2**) under identical conditions to those used for (**2**) gave, for unexplained reasons, a low yield of vancomycin with poor incorporation. However, the observed enhancement of the ^{13}C{^{1}H} signals for P6 and P8 is much lower in this sample, relative to α4 and α5, than for the vancomycin obtained when (**2**) was fed. This indicates that ^{2}H is present at P6 and P8 which is consistent with the retention mode for β-hydroxylation.

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