Biochemical Pharmacology, Vol. 37, No. 1, pp. 133–141, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

MOLECULAR BASIS OF THE ACTIVITY OF ANTIBIOTICS OF THE VANCOMYCIN GROUP

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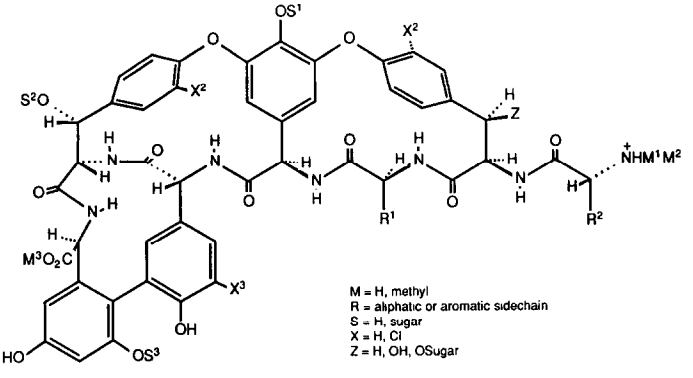
Antibiotics of the vancomycin group have assumed increasing clinical importance during the last fifteen years, in part because of the increasing prevalence of *Staph. aureus* bacteria which are resistant to methicillin [1]. In addition, vancomycin itself has found extensive use in the treatment of post-operative diarrhoea, caused by *Clostridium difficile* in the gut. The antibiotic is then given orally, and has been found to be very efficient in curing a dangerous condition.

As the importance of the antibiotics in this group has increased, pharmaceutical companies in many parts of the world have initiated efforts to find new members. As a result, the group now consists of a large number of structures, all of which are heptapeptides. For a vancomycin group antibiotic produced by any one actinomycete, there are often a number of variants, frequently differing in the nature of the attached sugars or fatty acid groups. The total number of structural variants which have been reported is in the region of one hundred; these variants can be encompassed by the general structure **1** (see, for example, Ref. 2).

For a number of years now, we have been engaged not only in the structure elucidation of members of this group, including the structure elucidation of vancomycin itself, but also in work designed to establish the molecular basis of their mode of action. The latter part of the work has been reliant on a finding [3] that vancomycin itself (**2**) and another member of the group, ristocetin A (**3**), bind to cell wall mucopeptide precursors terminating in the dipeptide -D-Ala-D-Ala. Given this knowledge, we examined the proton magnetic resonance spectra of



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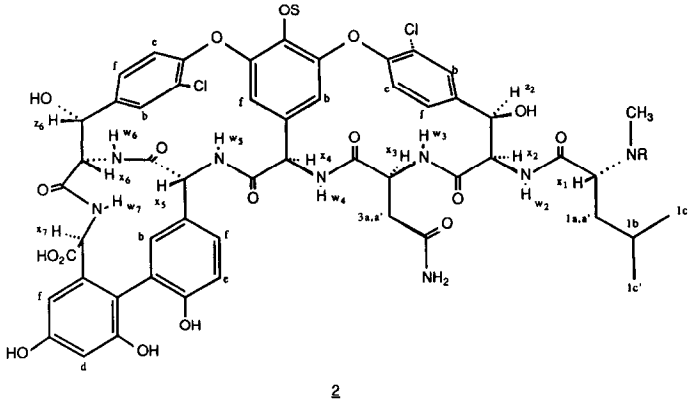

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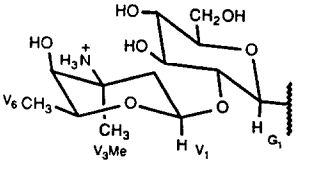

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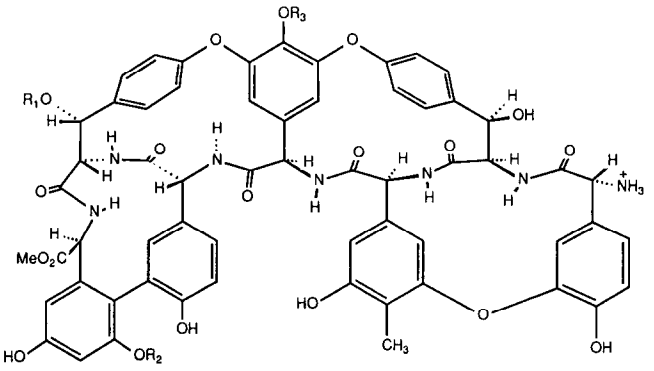

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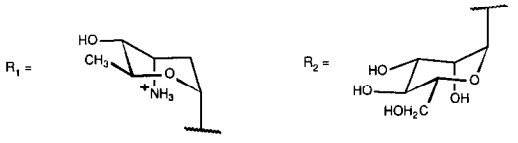
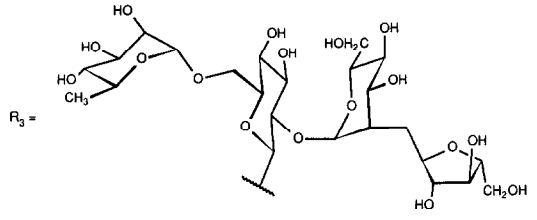




ristocetin A in both the presence and absence of the cell wall analogue *N*-acetyl-D-Ala-D-Ala [4]. Proton resonances of the antibiotic in its free form, and when bound to the cell wall analogue were assigned to specific protons in the structure. A similar analysis was carried out for the proton resonances of the peptide. With this information in hand, we therefore knew the changes in chemical shift of each proton resonance upon formation of a complex between the antibiotic and the cell wall analogue. In particular, the changing chemical shift of NH-protons of the antibiotic and cell wall analogue could be used to indicate which of these protons, when they occur as part of a secondary amide unit (—CO—NH—), are involved in hydrogen bond formation.

This information can be accommodated by proposing that in the complex between the antibiotic and the cell wall analogue, the cell wall analogue is oriented relative to the antibiotic as shown in Fig. 1. In the figure, dotted lines indicate hydrogen bond formation between the carbonyl group of one component and an NH group of the other component. It can be seen that in the proposed complex, the carboxyl group of the C-terminal alanine of the cell wall analogue forms no less than three hydrogen bonds to three NH groups which lie in a pocket at one end of the antibiotic structure. The hydrophobic walls of this pocket are created using four of the phenolic sidechains of the constituent amino acids of the antibiotic. They are orientated such that solvent

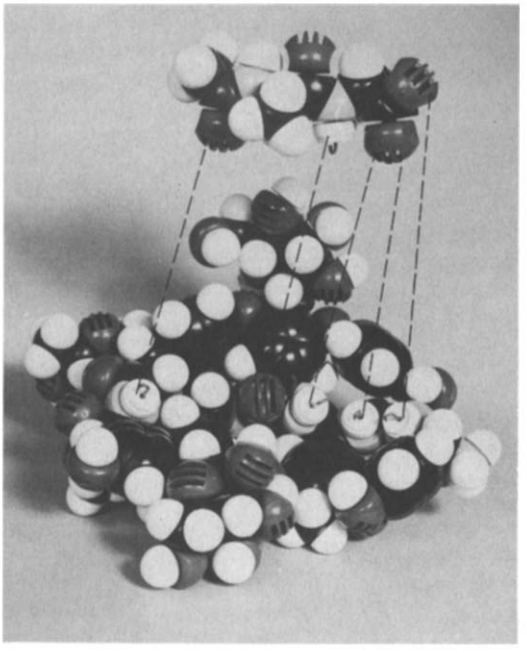


Fig. 1. A model of the interaction between ristocetin A (only the glucose unit of the tetrasaccharide is shown) and the bacterial cell wall analogue acetyl-D-Ala-D-Ala. The broken lines indicate intermolecular hydrogen bonds formed on complexation.

molecules may be excluded from the polar groups involved in the intermolecular hydrogen bonds of this region. As these interactions occur, an additional hydrogen bond can be formed between the carbonyl oxygen of the acetyl group of the cell wall analogue and an NH which is seen at the left hand part of the antibiotic structure. The geometry of these hydrogen bonded interactions allows simultaneously favourable hydrophobic interactions to occur between the two alanine methyl groups of the cell wall analogue and portions of the benzene rings of the antibiotic.

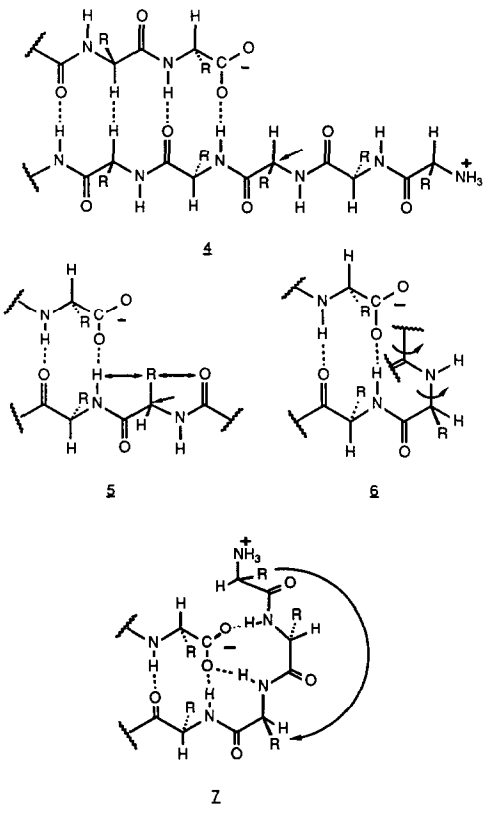
The above model for the binding interaction has been checked by use of the powerful technique of intermolecular nuclear Overhauser effects (nOes) [4b]. This is a means of determining the spatial proximity of hydrogen nuclei by observing their mutual relaxation towards equilibrium from an excited state created within the NMR experiment. The nOe data for the ristocetin–dipeptide complex showed that the binding model represented in Fig. 1 is indeed correct in its essential details. Those protons of the antibiotic and cell wall analogue which are demanded to be near in space by the binding model do give mutual nOes. Thus, the molecular basis of action of the antibiotics is well founded, and we have been investigating how these systems can be used to develop our understanding of molecular recognition.

Following earlier work [5], it has been possible to measure the binding constant between the two components by the use of UV-difference spectroscopy. These measurements have not only been carried out for numerous antibiotics of the vancomycin group with *N*-acetyl-D-Ala-D-Ala, but also to the extended cell wall analogue, di-*N*-acetyl-L-Lys-D-Ala-D-Ala. The binding constants in general lie in the range 10^4–10^7 l mol^{-1}, with the somewhat larger binding constants (by about 10^1–10^2) normally being found to the more extended cell wall analogue.

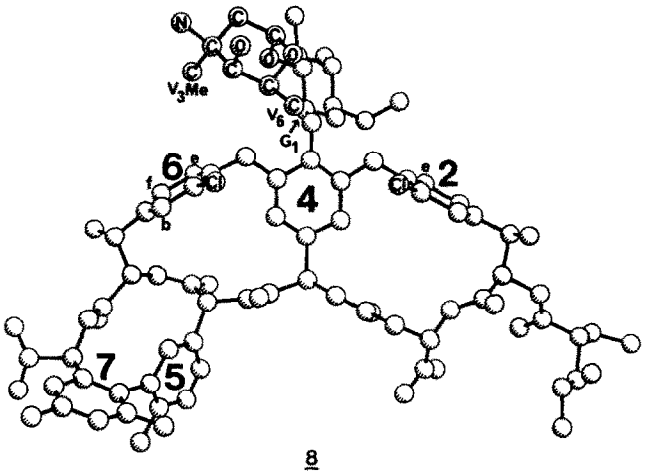
Given the above background information, in more recent work we have been able to explore some of the factors involved in defining the desired antibiotic geometries, and factors involved in more subtle aspects of binding the cell wall analogues. This paper is concerned with aspects of this work.

GENERATION OF THE CARBOXYLATE ANION BINDING POCKET

All members of the vancomycin group of antibiotics so far discovered exhibit in their bound state a carboxylate binding pocket whose most general feature is three inward pointing NHs, derived from three successive amides of a peptide backbone (the amides of amino acids 2, 3 and 4 from the N-terminus; see **1**). The nature of this pocket, and the three hydrogen bonds formed between it and the carboxylate anion of the cell wall analogue is seen in Fig. 1. A key question is "how are these three NHs made to point in the same direction, when normally in a poly-R or poly-S-peptide in its β-pleated sheet form they would alternate up/down/up (see **4**)?" In ristocetin, the cross-linkage of the sidechains of residues 1 and 3 of the antibiotic creates a barrier to the significant reorientation of the amide units of



residues 2, 3 and 4 that cannot be overcome at temperatures up to 100°. Thus, the conformations available to these amide units are restricted to ones closely related to the orientation shown in Fig. 1, which is presumably determined within the biosynthetic pathway of ristocetin. However, vancomycin for example, has no such restriction and so it is important for effective complexation that conformations other than the binding conformation are not strongly energetically favoured when no bacterial cell wall is present. We have concluded that the low energy requirement for pocket formation in vancomycin mainly derives from the fact that the amino acids have the *R*, *R*, *S* and *R* configurations at positions 1, 2, 3 and 4 from the N-terminus, i.e. residue 3 has the opposite absolute configuration to residues 1, 2 and 4 (see **1**). As a consequence, a β-pleated sheet form would contain the two repulsive 1,3-interactions indicated by double headed arrows in **5**. These interactions can be relieved by rotating the backbone to produce **6**, but the carbonyl oxygen atom which is omitted in **6** now has an unfavourable steric interaction with the NH group of amino acid 4. This interaction can in turn be relieved by rotating the amide bond which is bounded by arrows in **6** through about 180°. The conformation **7** results, and this is essentially the shape found in the carboxylate anion binding pockets of the antibiotics. Note that in **7**, the three inwardly pointing NH groups of amino acids 2, 3 and 4 can be constrained together if the sidechains of residues 1 and 3 are held approximately coplanar on the top side of **7** as presented, and the sidechains of residues 2 and 4 are similarly held



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approximately coplanar on the bottom side of 7. In fact, in all antibiotics of the vancomycin group so far discovered, residues 2 and 4 are indeed constrained near the same plane by covalent cross-linking of their sidechains (see, for example, 2, and 3). In ristocetin A (3), the sidechains of residues 1 and 3 are also covalently cross-linked.

In free vancomycin monohydrochloride in d_6-DMSO solution, nOes are observed between the amide NH (coded W_3) of the asparagine residue (residue 3) and the α-CH (coded X_2) of residue 2, the benzylic CH—OH (coded Z_2) of residue 2, and the aromatic ring proton (coded 2b) adjacent to chlorine in residue 2 (see Fig. 2, which shows the "back face" of vancomycin). These nOes establish that the amide proton of residue 3 spends some of its time on the back face of vancomycin, i.e. on the face shown in Fig. 2, which is the one hidden in conventional displays of the vancomycin group binding pocket (e.g. Fig. 1). Thus, these nOes show that in free vancomycin, a conformation is populated in which the carboxylate anion binding pocket is not present. However, W_3 also shows nOes to the amide NHs of residues 2 and 4 (coded W_2 and W_4), and to the aromatic ring proton (coded 2f) on the front face of residue 2 (see Fig. 3, which shows the front face of the vancomycin molecule). These nOes establish that a second conformation is populated, in which W_3 is on the front face of the structure, and the carboxylate anion binding pocket is indeed formed to an appreciable extent prior to binding the cell wall analogue. The interconversion of the two conformers requires the rotation, through approximately 180°, of the amide bond formed between the carbonyl of residue 2 and the NH of residue 3. This process is indicated schematically in Fig. 4.

The above process is slow on the NMR timescale, as deduced from the following evidence [6]. At room

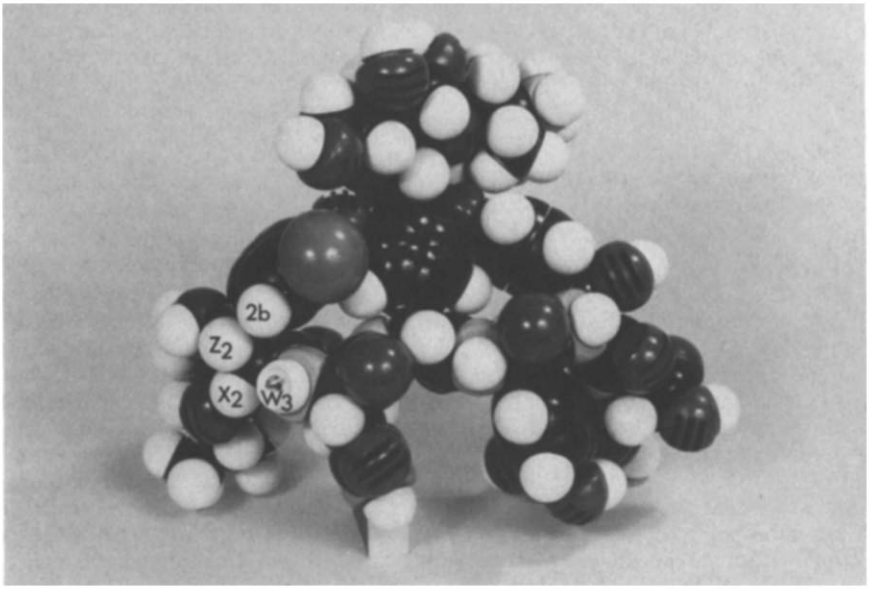

Fig. 2. The rear (non-binding) face of vancomycin showing one orientation of the W_3 amide unit, i.e. with the amide proton adjacent to protons X_2, Z_2 and 2b.

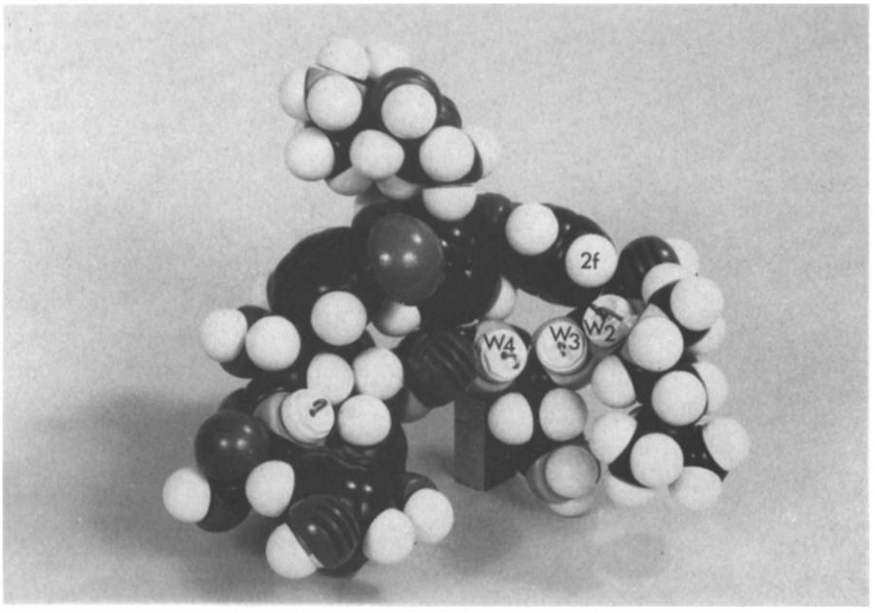

Fig. 3. The front (binding) face of vancomycin showing the second orientation of the W_3 amide unit, i.e. with the amide proton adjacent to protons W_2, W_4 and 2f.

temperature, the resonances of the amide NH protons W_2, W_3 and W_4, associated with residues 2, 3 and 4, are very broad compared with those (W_5, W_6 and W_7) of the other amide NHs (Fig. 5, spectrum obtained in d_6-DMSO solution). On heating the solution, the resonances of W_2 (initially at least), W_3 and W_4 sharpen, with those of W_3 and W_4 attaining approximately the same linewidth as those of W_5 and W_7 at about 350°K (Fig. 5). Above 310°K, the exchange of W_2 with traces of water in the solvent becomes the primary determinant of the linewidth of its resonance; this process is incidental to our

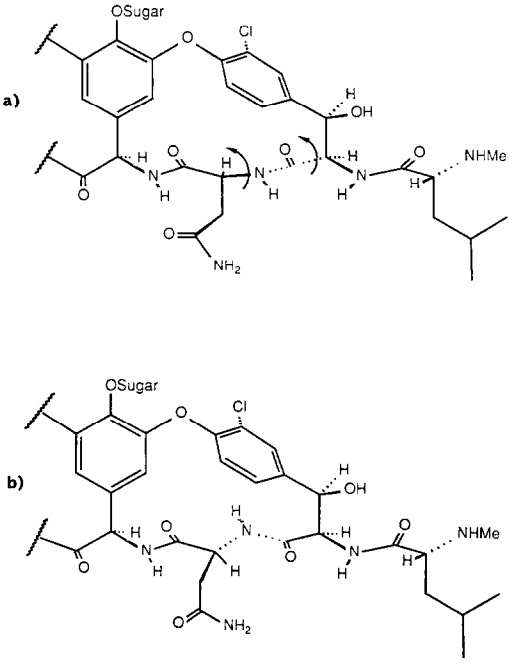

Fig. 4. A schematic representation of the rotation of the W_3 amide unit with the amide proton passing from the front face, as in (a), under ring 2 to the rear face, as in (b).

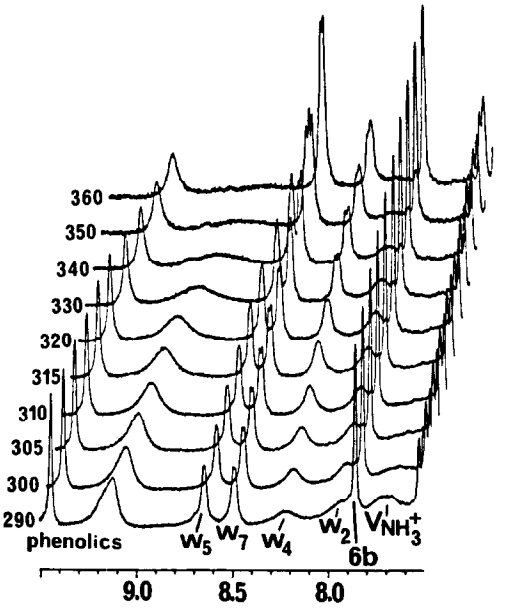

Fig. 5. A temperature profile (in °K) of the NMR resonances of selected amide protons of vancomycin, both affected and unaffected by motional/exchange phenomena. Also present are resonances of phenolic protons (in slow exchange with water in the solvent) and an aromatic proton.

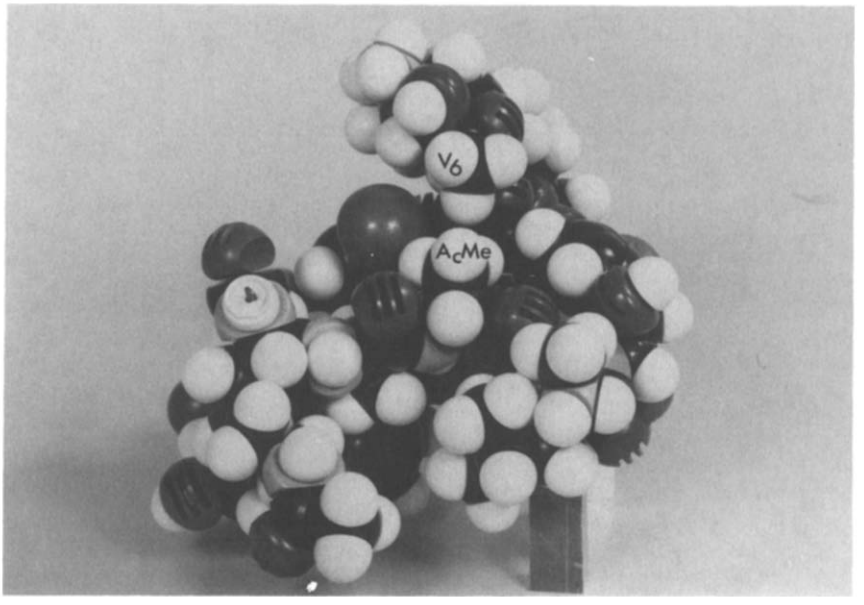

Fig. 6. A model of the complex formed between vancomycin and di-*N*-acetyl-L-Lys-D-Ala-D-Ala showing the spacial proximity of a hydrophobic region (V_6) of vancosamine and the C-terminal alanine methyl group (A_cMe).

present considerations, but is catalysed by the basic NH_2 group at the N-terminus of the antibiotic [6].* Similar, though less dramatic, sharpening is observed for the resonances of X_2, X_3 and Z_2 but not for other α-CH and β-CH protons. These selective sharpenings of the specified lines indicate a process which is passing from an intermediate exchange rate to the fast exchange limit as the temperature is increased. In the light of the above nOe evidence, and the fact that the selectively sharpened resonances are all due to protons in the vicinity of the amide bond connecting residues 2 and 3, we conclude that this amide bond is undergoing slow rotation. Estimates of the chemical shift differences which might be observed in the slow exchange limit lead us to conclude that the amide bond is rotating at a frequency in the range 200–1000 Hz [6]. Thus the barrier to the rotation must lie near to 12–14 kcal mol^{-1}.

The above conclusions may be of utility in the design of a carboxylate anion binding pocket. They indicate that an appropriate pocket might be found within a tetrapeptide unit if the residues (counted from the N-terminus) had *R*, *R*, *S* and *R* (or *S*, *S*, *R* and *S*) configurations, and an appropriate connection of the sidechains of residues 2 and 4. Such an arrangement can apparently destabilise a normal alternating backbone (see **4**) to such an extent that the free energies of three successive NHs "up-down-up" and "up-up-up" become comparable. It is moreover evident from our considerations that the desired "up-up-up" arrangement can be further stabilised by appropriate cross-linking of residues 1 and 3 (at the cost of slightly greater synthetic complexity). The synthesis of such pockets is in hand.

* In this connection, it is noteworthy that in vancomycin hydrochloride in d_6-DMSO solution, the amino group of the sugar vancosamine is protonated, but the terminal amino and carboxyl groups of the peptide backbone exist as neutral entities (rather than as the zwitterionic form which exists in water).

ROLE OF THE ANTIBIOTIC SUGARS

From the early work, it is evident that the major basis for the antibiotic–cell wall analogue interaction lies in the aglycone portion of the antibiotics. However, the antibiotics are found to carry 1 to 6 sugars. For example, the sugars glucose and vancosamine are found in vancomycin (**2**), and mannose, ristosamine, and a tetrasaccharide are found in ristocetin A (**3**). While it always appeared likely that these sugars would promote *in vivo* activity of the antibiotics by promoting their aqueous solubility, it is only now becoming evident that at least some of them aid binding in subtle and interesting ways.

(i) *The role of vancosamine in vancomycin*

When vancosamine is selectively removed from vancomycin, the binding constant to the cell wall analogue di-*N*-acetyl-L-Lys-D-Ala-D-Ala drops by a factor of about 3 [7]. Inspection of an X-ray structure (**8**) of a degradation product of vancomycin, CDP-1, immediately suggests a reason for this drop. In the X-ray structure, the 6-methyl group of vancosamine lies immediately next to the benzene ring of the amino acid residue 4 (see **8**); it is this benzene ring that undergoes a hydrophobic interaction with the methyl group of the C-terminal alanine residue of the cell wall analogue (see Fig. 6). Thus, the 6-methyl group of vancosamine can block the access of water to the methyl group of the C-terminal alanine residue, and so improve the overall hydrophobic interactions of the latter.

Table 1. Binding constants ($\times 10^3$) to peptides that model sites within bacterial peptidoglycan for vancomycin and ristocetin (taken from Ref. 12)

	Peptide	Vancomycin	Ristocetin
1.	Di-Ac-L-Lys-D-Ala-D-Ala	1500	590
2.	Di-Ac-L-Lys-D-Ala-D-Lys	14	100
3.	Di-Ac-L-Lys-D-Ala-D-Leu	9	610
4.	Ac-L-Ala-D-Glu-Gly	480	0.7

Further experiments show that although this idea is basically correct, the significance of the interaction is subtly modified by the presence of the NH_3^+ group of the amino-sugar vancosamine. Thus, acetylation of the amino group of vancosamine also reduces the binding constant to the same cell wall analogue by a factor of about 3 [7]. Determination of nOes when the cell wall analogue is bound to vancomycin, and when bound to vancomycin *N*-acetylated in the sugar residue, establishes that the 6-methyl group of vancosamine is adjacent to the methyl group of the C-terminal alanine residue in both complexes. Hence, we are forced to the conclusion that the associated hydrophobic interaction is strengthened (and indeed only contributes significantly to binding) in the presence of the charged amino group.

On reflection, the physical basis for such an effect is clear. Water molecules in the vicinity of the NH_3^+ group will be relatively strongly bound to each other, and relatively highly ordered. As a consequence, the penalty in free energy to be paid for disrupting this local water structure by a hydrocarbon surface will be larger than in the absence of the charge. Thus, the hydrophobic effect is strengthened by the proximate charge. A macroscopic expression of this kind of effect is found in the familiar "salting-out" phenomenon used in working up some organic reactions; a substance of limited hydrophobicity can be induced to pass from an aqueous to a non-polar phase by increasing the ionic strength in the aqueous phase. Of course, since the effect in the antibiotic is intramolecular, the local "salting out" is highly effective.

(ii) *The role of mannose in ristocetin A*

The removal of mannose from residue 5 (see (**1**)) of ristocetin A (**3**) reduces the binding to *N*-acetyl-D-Ala-D-Ala by slightly in excess of 1 kcal mol^{-1} (about a factor of 10 reduction in the binding constant) [8]. The molecular basis of this effect seems clear from nOe experiments [9]. The anomeric proton of the mannose residue shows an nOe to the methyl sidechain of the central alanine residue of the tripeptide cell wall analogue. Hence, it is concluded that a portion of this methyl group can be shielded from water by a relatively hydrophobic portion of the sugar residue.

However, the binding constant of ristocetin A to tripeptide is not changed significantly upon removal of mannose (and simultaneously of the tetrasaccharide) [10]. Thus, it must be concluded that although the mannose residue is important in shielding the methyl group of the non-C-terminal alanine residue from water in dipeptide, it has no such significant role when the tripeptide binds. This apparent anomaly is resolved by the observation that when the tripeptide binds to ristocetin A in aqueous solution, the lysine sidechain folds over the aromatic ring of residue 7.* Evidently, the lysine sidechain protects the methyl group of the non-C-terminal alanine from solvent to an extent that makes the corresponding role of mannose insignificant. It may additionally be that in the complex with tripeptide, the lysine sidechain and the mannose show an unfavourable steric interaction that prevents them from being able to shield the methyl group simultaneously and optimally from water. In this connection, it is noteworthy that vancomycin, which lacks the mannose residue, binds much more strongly to tripeptide (1.5×10^6) than to dipeptide (6.4×10^4).

(iii) *Specificity as a role for vancosamine and mannose*

The above results show that the contribution of both these sugars to the thermodynamics of binding of the relevant antibiotic and the bacterial cell wall precursor model di-*N*-acetyl-L-Lys-D-Ala-D-Ala is small or insignificant. However, their presence has a considerable influence on the effectiveness of each antibiotic against specific bacterial types. Previous to the structure determination of any members of the vancomycin of antibiotics, it was shown [12] that vancomycin strongly energetically disfavours a large sidechain in the amino acid at the C-terminus. Table 1 contains examples of this specificity (i.e. the replacement of the C-terminal D-alanine of the peptide with D-lysine or D-leucine, or the second D-alanine with D-glutamic acid), the relevance of which is discussed below. Although such specificity had been observed, it is only now that the role of the sugar substituents in creating this specificity, and their relevance to "nature's design" of these antibiotics has been recognised.

The NMR experiments discussed above have shown how in vancomycin, that vancosamine "caps" the binding pocket of the sidechain of the C-terminal amino acid of the peptide. Inspection of molecular models shows us that to avoid unfavourable interactions between a sidechain larger than a methyl group and vancosamine, it is necessary to significantly reorientate the sugar. Irrespective of the hydrophobic interactions involving the sugar known to exist during the occupation of this pocket by alanine (about 0.7 kcal mol^{-1}), the increase in energy as vancosamine crosses ring 2 or ring 6 (i.e. a rotation of about $\pm 90°$) is of the order of 5 kcal mol^{-1}, which

* This observation was initially made for avoparcin [11] and has subsequently been shown in our laboratory to be true also for ristocetin.

is comparable with the total binding energy. A 180° rotation of the disaccharide of vancomycin merely swops the positions of the vancosamine and glucose portions. Thus, in vancomycin, the unfavourable interactions of a large sidechain in the C-terminal amino acid of the peptide with the disaccharide attached to ring 4 of the antibiotic strongly energetically disfavour complex formation. By this means, specificity for peptides terminating in D-alanine is achieved.

A second component of the steric restriction to this pocket present in vancomycin but absent in ristocetin is the chlorine substituent of ring 6. However, studies involving the chemical replacement of this substituent with a proton revealed [13] that it alone is not the main source of the steric restriction to the top of the pocket. This does not rule out the possibility of a collaborative effect of the sugars and the chlorine substituent—indeed, in all known cases so far, whenever vancosamine (or another amino sugar) is attached as in vancomycin to ring 4 of the antibiotic, the ring 6 chlorine substituent is present.

Mannose attached to ring 7 of the antibiotic, as in ristocetin, performs an analogous role to that of vancosamine but the target amino acid in this case is the second from the C-terminus of the peptide. Again, the energy cost to move mannose from its solution conformation in the absence of peptide to a position that does not unfavourably interact with a sidechain larger than a methyl group is significant. Thus, specificity for peptides containing D-alanine as the second amino acid is achieved. It is likely that avoparcin achieves similar specificity to that of ristocetin through a rhamnose substituent of the *p*-hydroxyphenylglycine that constitutes the *N*-terminal residue of this antibiotic. Inspection of molecular models reveals that on formation of the peptide complex this sugar occupies a similar position to the ring 7 mannose substituent of ristocetin.

Why is such specificity required? Bacterial cell walls are biosynthesised from the inside and therefore to inhibit this process it is necessary for the antibiotic to pass through the cell wall currently in existence. Gram-positive bacteria with Type I cell walls (as classified by Ghuysen in Ref. 14) of which *Bacilli* species are a common example contain within each peptidoglycan cross-linkage the amino acid sequence shown in Fig. 7a. An antibiotic, such as ristocetin, that lacks specificity for a methyl group as the sidechain of the C-terminal amino acid of the peptide binds almost as strongly to this region as it does to the site of action. Although binding constant data for this peptide are not available, the comparable values of the binding constants to ristocetin of peptides 1 and 2 in Table 1 indicate the absence of selectivity against binding to peptides similar to those found throughout *Bacilli* cell walls. Thus, the mature cell wall peptidoglycan can act as an overwhelming source of decoy sites for antibiotic binding that shield the biosynthetically active interior. This ensures that the concentration of antibiotic that occurs at its site of action remains low—the cell wall effectively "buffering" the antibiotic concentration until its sites are saturated.

Comparison of the binding to vancomycin of peptides 1 and 2 in Table 1 shows how the selectivity created by the presence of vancosamine reduces the binding constant to peptides analogous to those in Type I cell walls by two orders of magnitude. Thus, the competition for antibiotic by intact cell wall is insignificant at the site of action, making the antibiotic more effective.

Gram-positive bacteria with Type III cell wall [14] (e.g. *Micrococci*) contain the peptide fragment shown in Fig. 7b within every peptidoglycan cross-linkage, i.e. a peptide with a large sidechain in the second amino acid from the C-terminus. Binding constants for vancomycin and ristocetin to a model of this peptide, *N*-acetyl-L-Ala-D-Glu-Gly, (see peptide 4 in Table 1) reveal that vancomycin binds to it only 3 times less effectively than to peptide 1, the model for the site of action. Ristocetin, in contrast, favours peptide 1 over peptide 4 by three orders of magnitude. For Type III bacteria, the cell wall effectively buffers the concentration of vancomycin

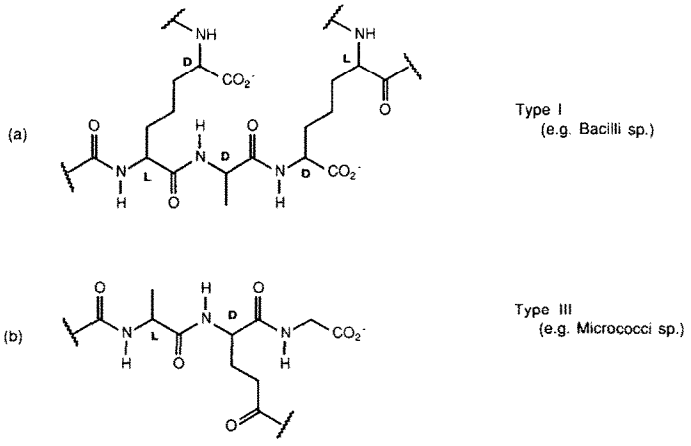

Fig. 7. Examples of peptides found within mature cell walls of (a) Type I bacteria, and (b) Type III bacteria, (see Ref. 14) that are stereochemically correct to bind to vancomycin group antibiotics. Note that in certain *Bacilli* species, e.g. *B. subtilis*, the free carboxylate anions shown in (a) are amidated and so no combination would be expected.

that reaches the site of action, but the selectivity partly imposed by the ring 7 mannose substituent of ristocetin vastly reduces the equivalent inhibition of this antibiotic.

Experimentally [15], a 50% inhibition of peptidoglycan synthesis in *Micrococcus lysodiekticus* occurred with 7.5 μg/ml of ristocetin compared with 20 μg/ml of vancomycin. More significantly, in a *M. lysodiekticus* cell-free system that synthesises peptidoglycan, the addition of a preparation of mature cell walls from the same bacteria completely reversed inhibition of the synthesis caused by vancomycin. Under the same conditions, only a 30% reversal of the inhibition caused by ristocetin was observed.

CONCLUSION

The formation of a carboxylate anion binding pocket in the antibiotics of the vancomycin group is made energetically more favorable by the *S* stereochemistry of residue 3 relative to the *R* stereochemistries of residues 1, 2 and 4. Pocket formation is further favoured by the covalent cross-linking of the sidechains of residues 2 and 4, and in some cases, also of residues 1 and 3. In the absence of the latter cross-link in vancomycin itself, the pocket is only partially formed in the free antibiotic, and is in equilibrium with a second conformation in which the peptide backbone alternates as in a β-pleated sheet.

The sugar vancosamine plays a small role in the thermodynamics of binding of vancomycin to cell wall analogues. This role is based upon a hydrophobic interaction between the 6-methyl group of the sugar and the methyl group of the C-terminal alanine of the cell-wall analogue. The hydrophobic interaction is however only significant in the presence of the nearby charged amino group of the sugar. It is believed that the charged group, by local ordering of the water structure, strengthens the hydrophobic interaction.

The sugar mannose in ristocetin A is able, by partially shielding the non-C-terminal alanine methyl group of the dipeptide cell wall analogue from water, to increase the binding of ristocetin A to this analogue. However, in the binding of the tripeptide cell-wall analogue, this same methyl group is shielded from water by the sidechain of lysine, and mannose loses a significant role in the thermodynamics of binding. This observation accounts for the much larger increase in binding, on passing from di- to tri-peptide, observed for vancomycin (which lacks mannose) relative to ristocetin A (which possesses mannose).

Both vancosamine and mannose contribute to antibiotic activity by improving the specificity of binding. The energetic cost to the necessary reorientation of the sugars away from sidechains larger than a methyl group in the target peptide diminishes binding to such peptides. Vancosamine, in vancomycin, applies this effect to the C-terminal amino acid of the peptide whereas mannose in ristocetin applies an analogous effect to the amino acid second from the C-terminus. Such selectivity prevents vancomycin from binding significantly to redundant peptides in the mature cell wall of, for example, *Bacilli* and ristocetin from the same fate in, for example, *Micrococci*. Thus, the lethal concentration of antibiotic for these bacteria is reduced in the presence of the sugars.

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