tional properties of the unusual 11-membered carbocycles.

Concluding Remarks

Where might this chemistry lead? Certainly, several of the problems that were encountered are now solved sufficiently for general applications. Methods for generation of sulfonium salts, ylides, and simple cyclic sulfides of virtually any desired size are optimized and well understood. On the other hand, there are many opportunities for further study in related areas.

Stereocontrol in medium and large rings is one possibility. So far, excellent selectivity has been observed in alkene epoxidations, osmylations, and reductions in our laboratory. Except for the enolate alkylations and enone 1,4-additions explored by Still et al., there have been no extensive studies on other reaction types.

In the thioaldehyde area there are also many options. We have just begun to appreciate the potential of this simple and highly reactive functional group for selective bond formation in complex surroundings. Virtually any thioaldehyde is easily generated, including some whose oxygen counterparts remain unknown.

Projects that include some element of total synthesis also encounter other, relatively mundane problems. We have learned to respect one of these well beyond initial expectations: there is still no reliable way to achieve the $CH_2SR \rightarrow COR^1$ transformation at any carbon oxidation state. Of course there are many "paper solutions" that work in simple systems, but the extrapolation from di-*n*-butyl sulfide to some of our macrolide precursors has proved to be long indeed. In such situations, the scientific value of total synthesis is clear, regardless of the target. Nothing else provides the motivation to compare old and new methodology in a realistic setting.

I express my appreciation to the many co-workes who have contributed their ideas, enthusiasm, and hard work to the projects described here. Thanks are also due to the National Institutes of Health and to the National Science Foundation for funding and to the University of Wisconsin for maintaining an atmosphere where research is enjoyable.

Structural Studies on Some Antibiotics of the Vancomycin Group, and on the Antibiotic-Receptor Complexes, by ^{1}H NMR

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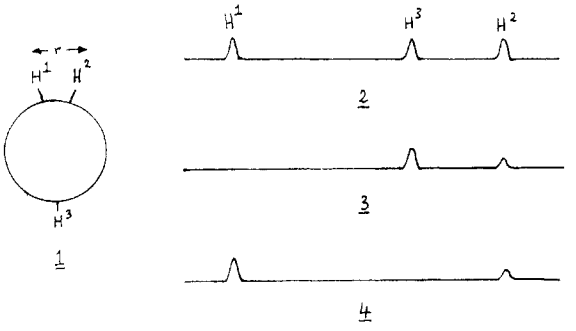
This Account is concerned with the story of the first structure elucidations of antibiotics of the vancomycin group (of vancomycin itself and of ristocetin) and with the discovery of the molecular basis of action of these drugs by binding to cell-wall analogues termining in -D-Ala-D-Ala. The work has been particularly rewarding, since in parallel with an increase in our understanding of the molecular basis of the drug action over the last decade, vancomycin itself has enjoyed a great increase in importance as a result of the occurrence of pathogenic strains of Gram-positive bacteria that are resistant to penicillin and cephalosporin therapy.

The success of the work is in large measure due to the use of the negative nuclear Overhauser effect (NOE), a phenomenon observed in nuclear magnetic resonance spectra. When nuclei which behave like bar magnets are placed in a magnetic field, they can occupy a high- and a low-energy state. A nuclear magnetic resonance absorption signal is obtained from such nuclei when, upon supplying electromagnetic radiation of a suitable frequency, ν, there is a net passage of nuclei from the low- to the high-energy state. Nuclei may pass back from the high- to the low-energy state by a process known as relaxation. Proton nuclei are normally relaxed by a mechanism which involves neighboring protons. The effectiveness of such neighboring protons in bringing about relaxation depends upon r^{-6}, where r is the internuclear distance between the proton being relaxed and the proton effecting the relaxation.

If the intensity of the resonance of one proton (H^1) is normally I, then if $r_{1,2}$ (the internuclear distance between protons H^1 and H^2) is relatively small (as a useful guide for our present purposes say, <0.3 nm), the effect of irradiating a second proton (H^2) before recording the intensity of the resonance of H^1 is to change its intensity to I'; i.e., $I \neq I'$. This change in intensity is called NOE. The effect is indicated schematically in 1–4. Three hydrogen nuclei in a molecule are indicated



Dudley H. Williams was born in Leeds, England, in 1937, and studied for his undergraduate and doctoral degrees at the University of Leeds. He subsequently worked at Stanford University as a postdoctoral fellow and then returned to the U.K. to carry out research and teaching at Cambridge University. He is a Fellow of Churchill College and Reader in Organic Chemistry at the University of Cambridge. His research interests cover the general areas of structure elucidation and mode of action studies on antibiotics. He has special interest in the development and application of new techniques in mass spectrometry and nuclear magnetic resonance. He is a Fellow of the Royal Society and a past recipient of the Meldola Medal of the Royal Institute of Chemistry, the Corday–Morgan Medal of the Chemical Society, and the Tilden Medal of the Royal Society of Chemistry.

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in **1**. The signals which arise in the ^1H NMR spectrum of **1** in the absence of preirradiating any of the protons are given in **2**. Upon preirradiation of H^1 at its resonance frequency ν_1, the ^1H NMR spectrum is modified to **3**. Note that in **3**, the resonance of H^1 has disappeared because its resonance is "saturated", i.e., the populations of upper and low energy levels are equalized, by the preirradiation. The H^2 resonance intensity is reduced due to the negative NOE. The NOE difference spectrum (NOEDS), **4**, is obtained by on-line computer subtraction of **3** from **2**. This Account is concerned only with negative NOES (usually found for molecules of molecular weights greater than ca. 1000), but positive NOES (intensity increases) are found for small molecules (molecular weights of, say, <500). Note that since H^3 is distant from H^1, no observable NOE occurs to H^3. The value of the difference spectrum is evident for molecules containing many protons; the NOEDS then contains only signals due to the irradiated proton H^1 and less intense signals due to those protons which are proximate to H^1. Such experiments are extremely valuable in determining structures of molecules and complexes in solution.

Structure Elucidation of the Vancomycin Group of Antibiotics

Vancomycin is a glycopeptide antibiotic obtained from *Streptomyces orientalis*, and it is bactericidal against Gram-positive bacteria. It was first isolated in 1956,[1] and now enjoys widespread clinical use, especially in the treatment of severe staphylococcal infections (e.g., endocarditis and wound septicaemia). A recent development has been its use orally in the treatment of pseudomembranous colitis, a severe and potentially lethal disease usually associated with antibiotic treatment after major gastrointestinal surgery.[2]

In the 1960s, methods suitable for the structure elucidation of vancomycin were not available, and early workers had to be content with the isolation of several important degradation products which retained the vast majority of the structure and with the characterization of *N*-methylleucine, aspartic acid, glucose, and chlorophenols, obtained as degradation products, each of which accounted for a small part of the structure.[3,4] However, with the advent of proton nuclear magnetic resonance at 270 mHz in the 1970s, it was possible to assign fully the ^1H NMR spectrum of vancomycin[5] in terms of all the structural units which had been isolated via chemical degradation.[6-9] Moreover, it was possible to assemble these units into a partial structure, largely by taking advantage of the negative nuclear Overhauser effect (NOE).[5] It is interesting to note that the discovery of these negative NOES (for vancomycin in

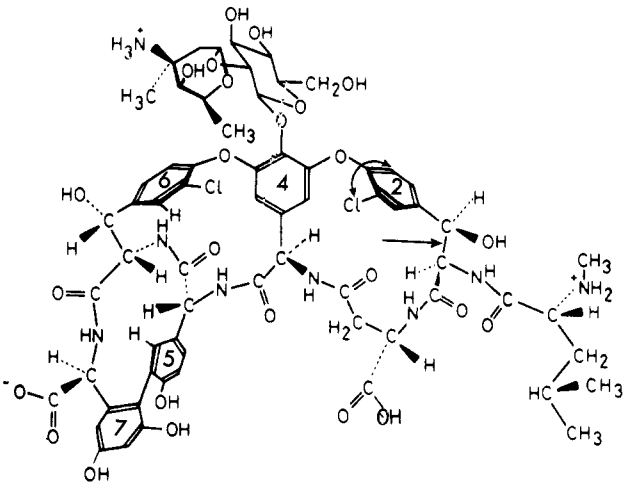

Figure 1. X-ray structure of the vancomycin degradation product, CDP-I.

Me_2SO-d_6) was purely serendipitous. In the process of assigning the spectrum by spin decoupling, it was noted that other resonances were reproducibly reduced in intensity. The work was being carried out by the author in 1976 while on sabbatical leave at the Department of Chemistry, University of Wisconsin, Madison. I did not immediately understand the origin of the effects. However, a conversation with Dr. P. Hart (Department of Pharmaceutical Chemistry, Madison) led to a consultation of the book by Noggle and Schirmer,[10] and attendance at a seminar[11] presented by Professor W. A. Gibbons on the campus clarified origins of the effects and led to their exploitation. It must be remembered that difference spectroscopy was not routinely available at this time, and hence positive NOES, which are normally small (1–5%), had not proved to be of wide utility and reliability. However, the negative NOES observed for vancomycin in Me_2SO-d_6 were large (15–50%), and hence readily observed without the use of difference spectroscopy.

The NOES are negative because vancomycin in Me_2SO-d_6 has a relatively slow tumbling time (ca. 10^{-8} s). This is due to a combination of its relatively large molecular weight (1448) and the use of a relatively viscous solvent. Despite the establishment of a partial structure,[5] there were too many asymmetric centers in the molecule to permit the determination of a complete structure. However, the early NOE work was crucial in showing the power of a method which has been used in establishing the structures of all other members of the vancomycin group.

The next important step for structure determination of the vancomycin group was the determination of the X-ray structure[12] of a degradation product of vancomycin, CDP-I.[3] The conversion to CDP-I is carried out by heating vancomycin at 80–90 °C in water at pH 4.2 for ca. 2 days. In this conversion a primary amide of vancomycin is converted to a carboxyl group. The X-ray structure of CDP-I is given in Figure 1. Since

(1) MCormick, M. H.; Stark, W. M.; Pittenger, G. F.; Pittenger, R. C.; McGuire, G. M. *Antibiot. Annu.* **1955–1956**, 606.
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(3) Marshall, F. J. *J. Med. Chem.* **1965**, *8*, 18.
(4) Johnson, C. R. Thesis, University of Illinois, 1962.
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(12) Sheldrick, G. M.; Jones, P. G.; Kennard, O.; Williams, D. H.; Smith, G. A. *Nature* **1978**, *271*, 223.

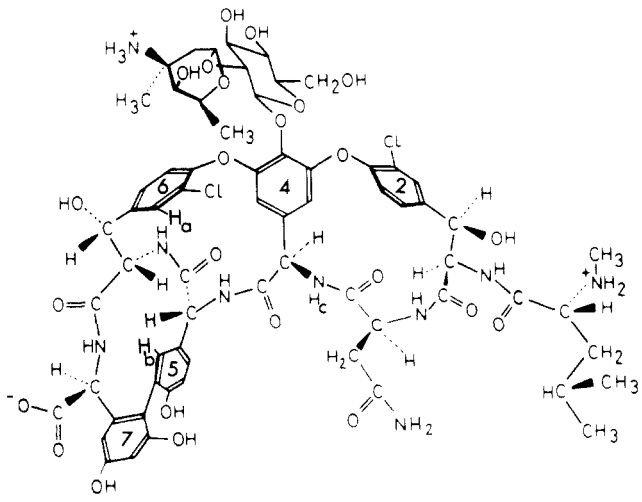

Figure 2. Structure of vancomycin.

earlier NMR studies[5] had established that the carboxyl group of the C-terminal meta-hydroxylated phenylglycine was free, it was assumed[12] that in vancomycin, the carboxyl group of the iso-Asp residue of CDP-I (Figure 1) was present as a primary amide, i.e., that vancomycin contains an isoasparagine residue and is otherwise the same as CDP-I.

The first evidence that the above assumption was incorrect came from further NOE studies carried out on vancomycin.[13] It might be asked why the further NOE studies were being carried out when it was assumed that the structure of vancomycin was known. The reason was that we had posed the hypothetical question: "Now that NOE difference spectroscopy was available, would it have been possible to elucidate the structure of vancomycin, with stereochemical detail, from ^{1}H NMR data without the help of X-rays?" Since the interpretation of the data obtained might at some point have been aided unconsciously by hindsight, it is even now difficult to answer the question unequivocally. However, two important points were clear.[13] First, certainly a great deal of the structure could be put together with complete stereochemical detail in agreement with that found in the X-ray of CDP-I (Figure 1). Second, in contrast to Figure 1, NOES showed that the chlorine atom attached to the ring labeled 2 in Figure 1 is in fact at the back of the molecule (see double-headed arrow in Figure 1) in vancomycin. Thus, this ring has rotated by ca. 180° in going from vancomycin to the crystalline CDP-I. If this were an equilibrium process, it might be regarded as a process of little significance. However, CPK models suggested that rotation of ring 2 would have been impossible at the temperatures used for the conversion of vancomycin to CDP-I. It was therefore concluded that bond fission (and re-formation) in the 17-membered macrocycle containing ring 2 (Figure 1) had occurred during CDP-I formation. This could be accommodated[13] by a reversible retro-aldol reaction which serves to break and remake the bond indicated by an arrow in Figure 1. However, Harris and Harris noted that it is not clear why such a process should occur with retention of the stereochemistry at the carbon atoms at the site of the proposed bond cleavage. They carried out ex-

(13) Williamson, M. P.; Williams, D. H. *J. Am. Chem. Soc.* **1981**, *103*, 6580.

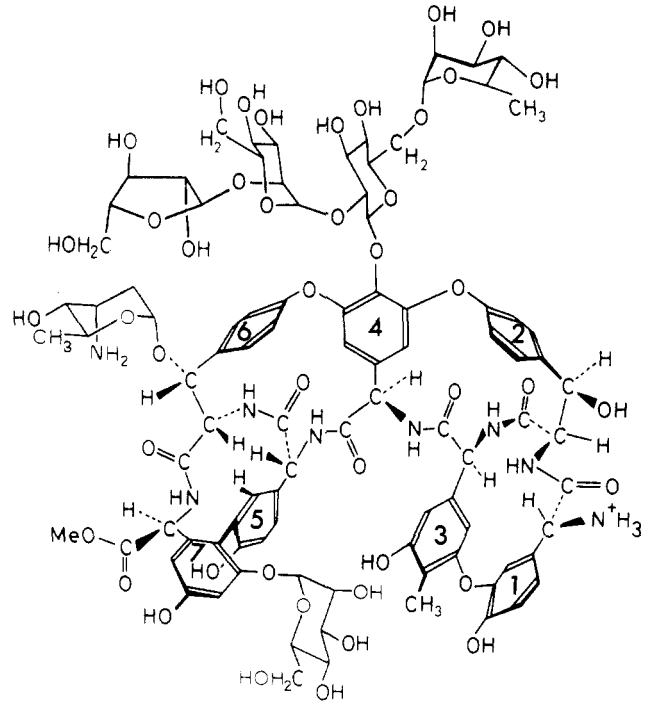

Figure 3. Structure of ristocetin A.

periments which showed[14] that vancomycin (Figure 2) contains an asparagine rather than an isoasparagine residue. At pH 4.2, asparagine isomerizes to isoaspartic acid via a substituted succinimide intermediate. Whereas the chlorine-containing ring 2 is indeed unable to rotate in vancomycin (Figure 2, containing the 16-membered macrocycle), it can do so slowly in CDP-I (Figure 1, containing the 17-membered macrocycle). Note that in CPK models both these rotations are utterly impossible, and in this respect the CPK models are misleading.

The early ^{1}H NMR work[5] and X-ray study[12] on vancomycin led to a situation where, using chemical and ^{1}H NMR methods alone, it has been possible to elucidate the structures of several other members of the vancomycin group. These include ristocetin,[15,16] avoparcin,[17] antibiotic A35512,[18] and actaplanin (A-4696).[19] All the antibiotics are linear heptapeptides (cf. Figure 2), and the unusual amino acid residues are linked together by several phenol oxidative couplings. The structure of ristocetin A,[15,16] in the determination of which chemical experiments were heavily complemented by ^{1}H NOES, is reproduced in Figure 3. Ristocetin is like all other members of the group so far discovered, except vancomycin, in containing seven aromatic rings. All the compounds share identical ox-

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ygen substitution patterns on rings 4, 5, 6, and 7. The stereochemistry at the asymmetric centers associated with the seven amino acids is conserved, and from the N-terminus is *R,R,S,R,R,S,S*.

Biosynthetic studies have been carried out on vancomycin[20] and ristocetin A.[21] It was found that both of the β-hydroxychlorotyrosine units of vancomycin (Figure 2) and both the β-hydroxytyrosine units of ristocetin A (Figure 3) can be derived from tyrosine. It was also established that the *p*-hydroxyphenylglycine units (numbering amino acid residues 1–7 from the N- to the C-terminus) at positions 4 and 5 in vancomycin and at positions 1, 4, and 5 in ristocetin A can be derived from tyrosine. The *m*-dihydroxylated phenylglycine units (residue 7 in vancomycin, and residues 3 and 7 in ristocetin A) are derived from acetate. The aromatic methyl group of residue 3 in ristocetin A is presumably derived from *S*-adenosylmethionine, but this has not been established experimentally. The polycyclic structures are formally completed from a heptapeptide by three phenol oxidative couplings (two C→O and one C→C) in vancomycin and four (an additional C→O) in the case of ristocetin A and by the addition of sugars. However, the order of these steps has not been established.

Molecular Basis of Action of the Vancomycin Group. With the above structures established, it was possible to probe the molecular basis of action of the antibiotics. The vancomycin group of antibiotics are inhibitors of bacterial cell-wall biosynthesis. A key finding was that vancomycin forms strong complexes with the cell-wall peptidoglycan precursor UDP-*N*-acetylmuramyl-pentapeptide.[22] By stepwise degradation of such a precursor, Perkins[23] was able to show that an acyl-D-alanyl-D-alanine C-terminus is the feature essential for complex formation. The molecular basis for complex formation was therefore probed by comparing the proton NMR spectra of the cell-wall analogue *N*-Ac-D-Ala-D-Ala, and of the antibiotics, when obtained alone, with those of the *N*-Ac-D-Ala-D-Ala/antibiotic complexes. The first study in this area[24] was carried out with the complex *N*-Ac-D-Ala-D-Ala/vancomycin before the structure of the antibiotic was established. Although few specific conclusions could be reached, therefore, this study was important in establishing the idea of using ^{1}H NMR in this way and in showing that the C-terminal alanine methyl resonance of *N*-Ac-D-Ala-D-Ala is shifted to high field by ca. 0.9 ppm upon complex formation. Thus, it was concluded that this methyl group lay over the face of a benzene ring of the antibiotic in the complex.

The later ^{1}H NMR work[5] on the structure of vancomycin had also reported details of the ^{1}H spectra of an equimolar mixture of vancomycin and *N*-Ac-D-Ala-D-Ala. Since the 1:1 complex was in fast exchange with the free components at room temperature in Me_2SO-d_6 solution, it was possible to follow the shifts of a number of the antibiotic protons upon complex formation. In particular, protons H_b and H_c underwent marked downfield shifts, and H_a underwent a marked upfield shift (Figure 2). It was therefore concluded[12] that these three protons, which are in a cleft on one side of the antibiotic, are near to the peptide fragment in the antibiotic–peptide complex and that NH_c is hydrogen bonded to a peptide carbonyl group. Furthermore, this carbonyl group is likely to be that of the carboxyl terminus of the peptide since, when this interacts with NH_c, the methyl group of the C-terminal D-alanine can be over the face of benzene ring 4 (Figure 2), thus accounting for the data of ref 24 (see above). When these interactions are optimized, the models indicate clearly that, simultaneously, the two additional hydrogen bonds shown in Figure 4 can be made.

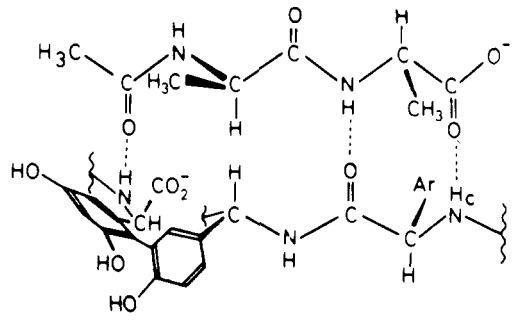

Figure 4. Interaction between *N*-Ac-D-Ala-D-Ala and a portion of the vancomycin molecule.

These interactions also cause the secondary methyl group of the N-terminal D-alanine to be over the benzene rings of the biphenyl moiety to give a favorable hydrophobic interaction. These proposals, based on the X-ray structure of CDP-1, have stood the test of time, and are all retained in currently accepted models of the binding. We shall see later, however, that they are complemented by further favorable interactions. These further interactions could only be deduced after it was subsequently shown that a portion of the vancomycin molecule, when bound to the cell-wall analogue, is in a conformation different to that found in the X-ray of CDP-I.

Before this conformational change was uncovered, a more complete binding site of ristocetin A for *N*-Ac-D-Ala-D-Ala was deduced.[25] This was possible because, at room temperature in Me_2SO-d_6 solution, the ristocetin A/*N*-Ac-D-Ala-D-Ala complex is in slow exchange with the free components on the ^{1}H NMR time scale. Therefore, it was possible, using techniques such as saturation transfer, to correlate the chemical shifts of many of the protons on passing from the free to the bound state. In particular, it was possible to do this for all the NH protons of the secondary amides in both components. This is important since all those NH protons which suffer large downfield shifts upon complex formation are clearly involved in hydrogen-bond formation. Additionally, it was possible to determine the temperature dependence of the chemical shifts of these same NH protons before and after binding. Using the criterion that those amide NHs that show a large temperature dependence ((6–10) × 10^{-3} ppm/°C to high field with increasing temperature) are solvent exposed, whereas those that show a low temperature dependence (0–3 × 10^{-3} ppm/°C) are partially or totally hidden

(20) Hammond, S. J.; Williamson, M. P.; Williams, D. H.; Boeck, L. D.; Marconi, G. G. *J. Chem. Soc., Chem. Commun.* **1982**, 344.
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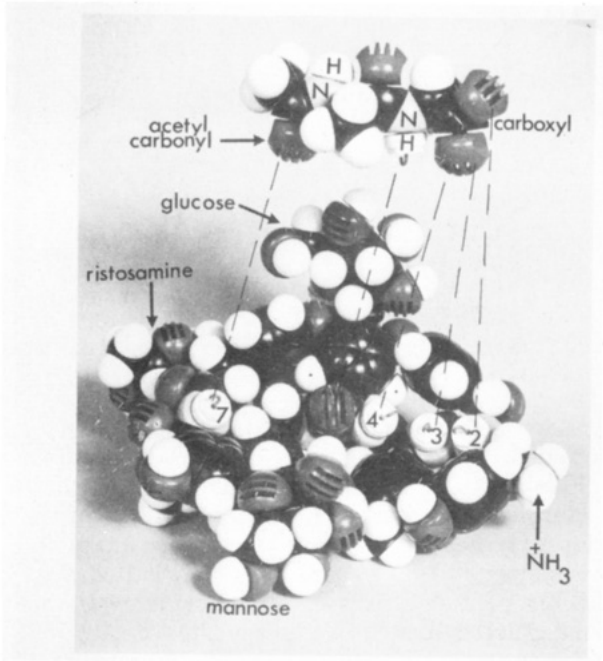

Figure 5. Molecular basis for the binding of *N*-Ac-D-Ala-D-Ala to ristocetin A. For clarity, in the ristocetin A structure only the glucose residue of the tetrasaccharide unit is shown. Hydrogen-bond interactions are indicated by broken lines.

from solvent, much about the mode of binding could be deduced.

First, the temperature dependence studies showed that the NH of the C-terminal D-alanine is hidden from solvent after the *N*-Ac-D-Ala-D-Ala binds, but that of the N-terminal D-alanine is still exposed after binding. Second, the data for the six amide NH's of ristocetin A divide them clearly into two groups. One group (two NH protons) is exposed to solvent before and after binding, and these two protons are on the "back" (as conventionally displayed), convex face of the antibiotic. The second group, of four NH protons, is located at the "front" of the molecule, is located in a cleft before binding (the NH's are partially hidden from solvent), and is clearly involved in hydrogen-bond formation in complexation and/or is even more hidden from solvent after the complex is formed than before.

These experiments established which side of the cell-wall analogue binds to which side of the antibiotic and give, with some help from the earlier work on the vancomycin mode of action, a picture for the molecular basis for complex formation (Figure 5). The main features of the binding are (i) a carboxylate binding pocket in ristocetin A, consisting of NH's 2, 3, and 4, which are in a cleft and flanked by hydrophobic walls constituted from the aromatic rings of residues 1 and 2, and the carboxyl group of *N*-Ac-D-Ala-D-Ala binds into this pocket by hydrogen-bond formation between its oxygen atoms and the NH groups 2, 3, and 4. Note that these hydrogen bonds, which are essentially formed due to electrostatic attractions [$>C{=}O^{\delta-}\cdots{}^{\delta+}HN<$], should be strengthened in a hydrophobic pocket which provides an environment of low dielectric constant. (ii) The binding of the carboxylate anion into its pocket is strengthened by the favorable Coulombic attraction between the $-CO_2-$ of the cell-wall analogue and the $-NH_3^+$ at the N-terminus of the antibiotic. (iii) The NH of the C-terminal D-alanine of the cell-wall analogue forms a hydrogen bond to the carbonyl group of residue 4 of the antibiotic; this carbonyl group can be seen at the center of the antibiotic structure shown in Figure 5. (iv) The NH-7 of the antibiotic forms a hydrogen bond to the carbonyl oxygen of the *N*-acetyl group of the cell-wall analogue. (v) When the above five hydrogen bonds are made, the methyl group of the C-terminal alanine (hidden in Figure 5) forms a hydrophobic bond into the face of the aromatic ring of residue 4 of the antibiotic (this is the benzene ring whose protons are marked by a dot in Figure 5) and the methyl group of the other alanine residue forms a hydrophobic bond over the benzene rings of residues 5 and 7, which constitute the biphenyl moiety.

The above details of the binding site are supported by intra- and intermolecular proton NOES determined in the complex.[25] Thus, the intramolecular NOES observed between protons of *N*-Ac-D-Ala-D-Ala when in the bound state indicate its conformation in the bound state. This conformation is essentially the same as, or very similar to, that found[26] in an X-ray structure of *N*-Ac-D-Ala-D-Ala. It is concluded that the cell-wall analogue binds to the antibiotic in the conformation which is likely to be close to that corresponding to an energy minimum. Four intermolecular NOES observed between protons of the antibiotic and of the cell-wall analogue[25] are those anticipated to occur when the two components shown in Figure 5 are brought into contact in the manner indicated in that figure. Note that intermolecular proton NOES are potentially a very powerful method for the determination of the molecular basis of the binding of a drug to its receptor. It is however limited by our inability to analyze the proton spectra of very large molecules. Two-dimensional methods are improving the situation dramatically, with complete assignments now being possible up to molecular weights of ca. 6000, and partial and useful assignments being possible at much higher molecular weights.

The efficiency of the above interaction, involving one small- and one medium-sized molecule, is remarkable. In light of this, there can be little doubt that the organisms which produce antibiotics of the vancomycin group do so because of the selectional advantage they then derive in competing with bacteria which can be attacked by binding to cell-wall precursors terminating in -D-Ala-D-Ala. Given these thoughts, we were led to bonder why the NH groups of residues 1, 2, and 3 in the X-ray structure of CDP-I (Figure 1) were so distant from the NH of residue 4 as to make it impossible to involve these in binding the cell-wall analogue (hence, the lack of their utilization in Figure 4). Even prior to these thoughts, Convert et al.[27] had noted that the model proposed in Figure 4 does not offer an understanding for the increased binding of *N*-Ac-D-Ala-D-Ala to vancomycin when its *N*-methylleucine residue is in its cationic (as opposed to its neutral) state.[24,28] To overcome this problem, they proposed[27] that the positively charged *N*-methylamino group approaches the D-Ala carboxylate ion more closely (~5 Å, contrasting with ~9 Å using the X-ray structure of CDP-I in con-

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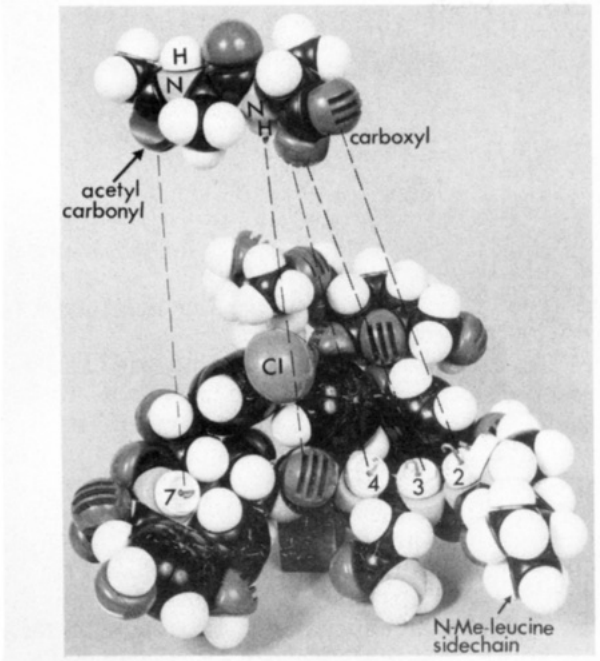

Figure 6. Molecular basis for the binding of *N*-Ac-D-Ala-D-Ala to vancomycin. Hydrogen-bond interactions are indicated by broken lines.

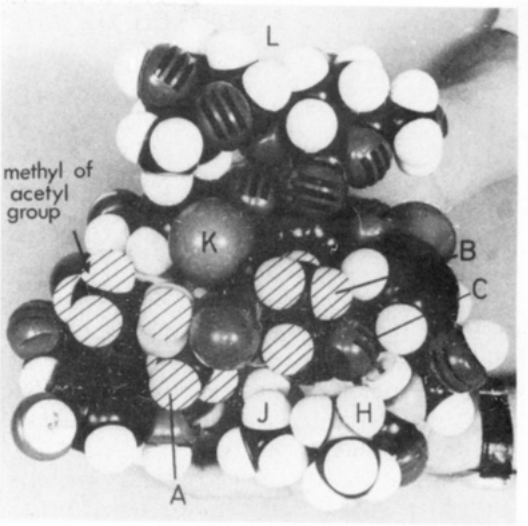

Figure 7. The vancomycin/*N*-Ac-D-Ala-D-Ala complex, viewed from above a cleft which binds the cell-wall analogue. Protons of cell-wall analogue are indicated by hatching. (A and B) Terminal and C-terminal methyl groups, respectively, of D-alanine residues; (C) an oxygen atom of the carboxylate anion of the cell-wall analogue; (H) $CH_3NH_2^+$ group of *N*-methylleucine; (J) methyl group of the *N*-methylleucine side chain [$(CH_3)_2CH_2CH$–]; (K) chlorine atom at the "front" face of vancomycin; (L) disaccharide unit of vancomycin.

junction with the interactions shown in Figure 4) in the complex, by invoking a conformational change involving the *N*-methylleucine residue. This proposal was supported by experimental evidence[27] but still leaves a proposed binding site which is far less sophisticated than that found for ristocetin A (Figure 5).

The portion of the vancomycin molecule involving residues 1, 2, and 3 is, however, conformationally mobile. It is possible to manipulate these residues so that a carboxylate-binding pocket, resembling that found for ristocetin A (Figure 5), is formed.[29] Evidence that such a carboxylate-binding pocket exists in vancomycin in its bound state was found by studying the vancomycin/*N*-Ac-D-Ala-D-Ala complex when in slow exchange with free components (achieved by working near 0 °C, using added CCl_4 as an "antifreeze" for Me_2SO-d_6),[29] and by employing NOE difference spectroscopy under these conditions.[30] The binding site which has been deduced as a result of these experiments is reproduced in Figure 6. It is sufficiently similar to that of ristocetin A not to merit detailed discussion. However, note that in the complex (Figure 7, bound view), the "fatty" side chain of *N*-methylleucine is folded back along the structure so that it provides one of the hydrophobic walls of the carboxylate ion-binding pocket. In this respect, the *N*-methylleucine $CH_2CH(CH_3)_2$ group replaces the benzene ring which is found in residue 1 in the ristocetin A structure (Figure 3).

Conclusion

The results that have been discussed show that ^1H NMR has increased enormously in power for structure elucidation of complex molecules since the advent of nuclear Overhauser effect difference spectroscopy. Equally important, it is clear that intermolecular NOES are likely to prove to be a generally powerful tool for determining the molecular basis for many interactions in solution which occur in biological systems. The system upon which we worked, namely, antibiotic to cell-wall peptide interactions, was chosen because the ^1H spectra of the resulting complexes could be almost completely analyzed. However, as has been demonstrated in recent years by other groups, the advent of 2D-NMR is making the spectra of ever-larger molecules amenable to extensive or total analysis.[31] Particularly useful in this respect are the two-dimensional correlations for homonuclear (in this case, proton) spin systems known as COSY,[32] SECSY,[33] and NOESY.[34]

In the 1970s, there was considerable justification for the view that, for complex biological structures, NMR spectroscopy in many cases merely gave support, in solution, for structures previously established by X-ray methods. This is certainly no longer the case; NMR is now making extremely important contributions in its own right, and appears likely to do so to an even larger extent in the future.

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