Nevertheless, it is difficult, at present, to assess the biological relevance of some of the reported results, e.g., whether Pt(G-H)≡G pairing is sterically possible in native DNA.

One final aspect should be mentioned. In connection with the mutagenic effects of modified nucleobases, the possibility of base mispairing caused by base ionization has been discussed.[50] Provided such a mechanism works, Pt-G complexes should be regarded likely candidates for it.

Acknowledgment. The author wishes to thank the Deutsche Forschungsgemeinschaft and the Technische Universität München for financial support, and one of the referees for kindly making available some references on relevant work he had not been aware of.

Supplementary Material Available: Tables of chemical shifts, graphs, and ^{1}H NMR spectra (17 pages). Ordering information is given on any current masthead page.

(50) Pullman, A. "Electronic Aspects of Biochemistry", Pullman, B., Ed.; Academic Press: New York, 1964; pp 135–152.

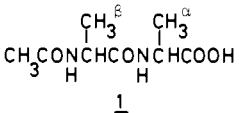
Binding Site of the Antibiotic Vancomycin for a Cell-Wall Peptide Analogue

Dudley H. Williams* and David W. Butcher

Contribution from the University Chemical Laboratory, Cambridge CB2 1EW, United Kingdom. *Received January 19, 1981*

Abstract: The previously proposed binding site of the antibiotic vancomycin for a cell-wall peptide analogue, Ac-D-Ala-D-Ala, while correct in the interactions postulated, omits further important interactions. It is shown that, relative to the conformation of vancomycin inferred from the X-ray structure of CDP-I, bound vancomycin has undergone a major conformational change involving isoasparagine, *N*-methylleucine, and the β-hydroxychlorotyrosine unit located between these two amino acids. As a result of the conformational change, vancomycin can form two further hydrogen bonds, as well as a salt bridge, in binding the cell-wall peptide analogue. The binding pocket for the carboxylate anion of Ac-D-Ala-D-Ala which is so formed closely resembles that found in ristocetin A.

Vancomycin is a clinically important antibiotic whose structure was determined by X-ray analysis in 1978.[1] Due to adverse side effects (tolerable, however, in serious illness), its use has been restricted to the treatment of staphylococcal infections (for example, wound septicaemia and pneumonia) when other antibiotics are ineffective. However, recently it has been reported that vancomycin is effective in the treatment of postoperative diarrhea; it causes the disappearance of *Clostridium difficile*, which may otherwise lead to potentially lethal complications following earlier treatment with other antibiotics.[2] Vancomycin acts by inhibiting the synthesis of cell-wall mucopeptide, which results in the eventual destruction of the cell by lysis. Nieto and Perkins have shown[3] that it forms complexes with cell-wall precursors which terminate with D-alanyl-D-alanine at the carboxyl terminus of a peptide portion. On the basis of the X-ray structure and conformation of vancomycin, the shifts of a limited number of proton resonances in its ^{1}H nuclear magnetic resonance (NMR) spectrum upon addition of the cell-wall peptide analogue acetyl-D-alanyl-D-alanine (**1**)[4,5] and an examination of space-filling (CPK) models, it was possible to propose a structure for the noncovalently bonded complex formed between the antibiotic and cell-wall analogue.[1] We now report that this complex, although correct in the proposed interactions between the antibiotic and cell-wall analogue, omits further important interactions. We show that the complexed form of the antibiotic undergoes a major conformational change (relative to the X-ray structure) and in so doing generates an efficient carboxylate-binding pocket for C-terminal D-alanine.



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The structure of vancomycin, based on the X-ray structure and conformation of the derivative CDP-I[1] (in which the primary amide of isoasparagine in vancomycin is replaced by a carboxyl group), is reproduced in Figure 1. An exploded view of the earlier proposed[1] structure of the complex between Ac-D-Ala-D-Ala and vancomycin is reproduced in Figure 2. It can be seen that since the carboxyl terminus (marked "C" in Figure 2) of the terminal D-Ala is only hydrogen bonded to NH_{C4}, in this model the *N*-methylleucine, "right-hand" β-hydroxychlorotyrosine, and isoasparagine residues of vancomycin are redundant in binding the cell-wall analogue (Figures 1 and 2). Recently, Convert and co-workers[6] have noted that the proposed model (Figure 2) does not offer an understanding for the increased binding of Ac-D-Ala-D-Ala when its terminal carboxylate group is in the anionic state and the vancomycin *N*-methylleucine is in its cationic state.[3,4] This is because the complex shown in Figure 2 places the D-Ala carboxylate anion ~9 Å from the positively charged *N*-methylamino group (Figure 1).[6] To overcome this problem, they have proposed[6] that the positively charged *N*-methylamino group approaches the D-Ala carboxylate anion more closely (~5 Å) in the complex by rotating it about the arrowed bond in Figure 1

(1) Sheldrick, G. M.; Jones, P. G.; Kennard, O.; Williams, D. H.; Smith, G. A. *Nature (London)* **1978**, *271*, 223–225.
(2) Keithley, M. R. B.; Burdon, D. W.; Arabi, Y.; Alexander-Williams, J.; Thompson, H.; Youngs, D.; Johnson, M.; Bentley, S.; George, R. H.; Mogg, G. A. G. *Br. Med. J.* **1978**, 1667–1669. Tedesco, F.; Markham, R.; Gurwith, M.; Christie, D.; Bartlett, J. G. *Lancet* **1978**, 226–228.
(3) Nieto, M.; Perkins, H. R. *Biochem. J.* **1971**, *123*, 789–803.
(4) Brown, J. P.; Feeney, J.; Burgen, A. S. V. *Mol. Pharmacol.* **1975**, *11*, 119–125. Brown, J. P.; Terenius, L.; Feeney, J.; Burgen, A. S. V. *ibid.* **1975**, *11*, 126–132.
(5) Williams, D. H.; Kalman, J. R. *J. Am. Chem. Soc.* **1977**, *99*, 2768–2774.
(6) Convert, O.; Bongini, A.; Feeney, J. *J. Chem. Soc., Perkin Trans. 2* **1980**, 1262–1270.

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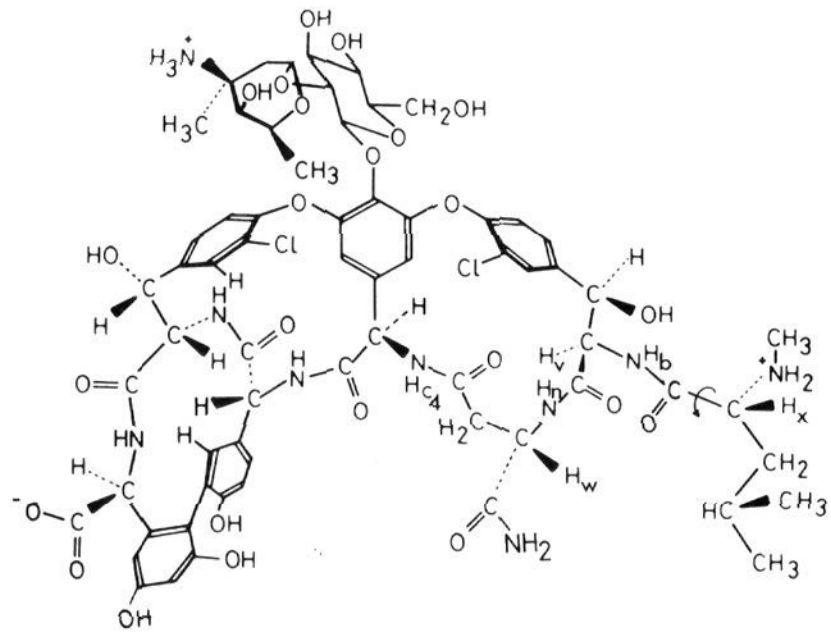


Figure 1. Structure of vancomycin, displayed in the conformation determined for crystalline CDP-I. The labeled protons (c_4, w, n, v, b, and x) are coded as in ref 5 and 6.

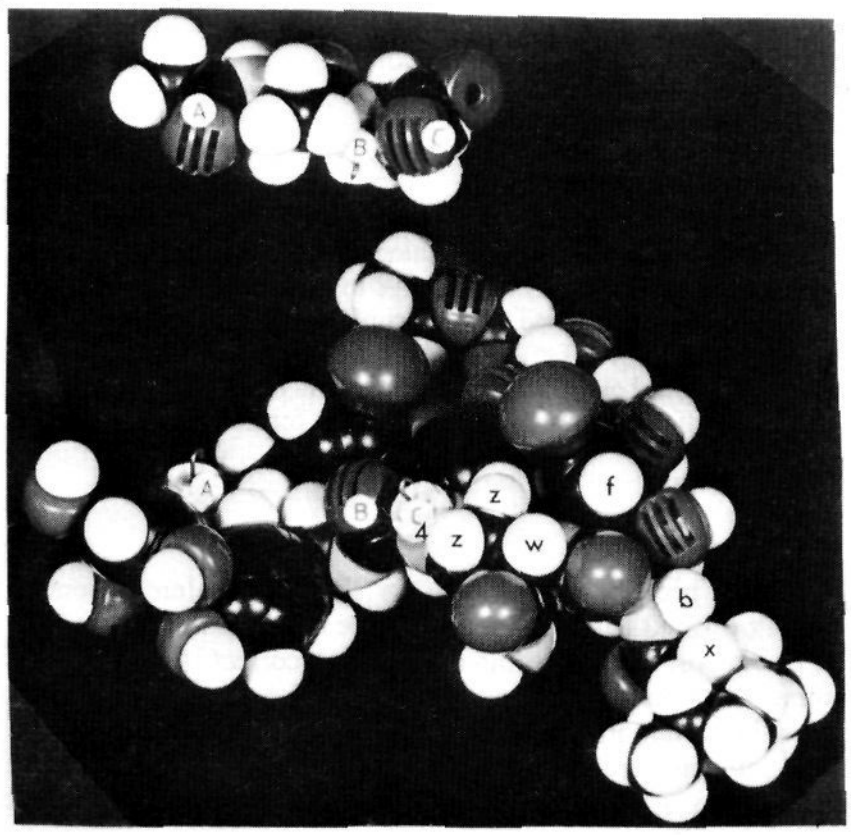


Figure 2. Exploded view of the previously proposed complex formed between vancomycin and the cell-wall peptide analogue Ac-D-Ala-D-Ala. The NH to C=O hydrogen-bonding interactions are A → A, B → B, and C → C_4.

by ~180°, while maintaining the position of the *N*-methylleucine carbonyl group constant. It will be seen subsequently that while our proposed complex also incorporates this change, it additionally makes fundamental changes to the shape of the peptide backbone of vancomycin, involving both the "right-hand" β-hydroxychlorotyrosine and isoasparagine residues.

We were guided in our approach to the structure of the vancomycin–cell-wall analogue complex by the recent elucidation of the structure of the complex formed between the antibiotic ristocetin A and Ac-D-Ala-D-Ala.[7] The latter problem proved to be soluble because the complex is in slow exchange with its separated components on the ^{1}H NMR time scale. Thus, the proton resonances of the secondary amides (–CON*H*–) in the structures can be assigned for both free and complexed components. Those amide proton resonances which shift markedly downfield upon complex formation are clearly involved in hydrogen-bond formation. This unequivocal approach had not been applicable in the past to the structure of the vancomycin–Ac-D-Ala-D-Ala complex, because in both aqueous media[4,6] and dimethyl sulfoxide solution[5,6] the complex was observed to be in fast exchange with its free components. We have overcome this problem by working with Me_2SO-d_6 solutions containing ~30% carbon tetrachloride. The mixed solvent is employed to (i) permit cooling of the solution to ~0 °C without freezing and therefore decrease the unimolecular rate constant for dissociation of the complex and (ii) possibly also decrease this rate constant by inhibiting the breaking of hydrogen bonds present in the complex in the more lipophilic medium.

(7) Kalman, J. R.; Williams, D. H. *J. Am. Chem. Soc.* **1980**, *102*, 906–912.

Table I. Chemical-Shift Changes of Some Proton Resonances of Vancomycin (Figure 1) and Ac-D-Ala-D-Ala (1) upon Complex Formation[a]

proton resonance	shift, Hz	proton resonance	shift, Hz
α (see 1)	+208	v	−186
β (see 1)	+43	x	−81
w	−81	b	−999

[a] The shifts are derived from the chemical shifts in the complex at −1 °C relative to those in vancomycin in Me_2SO at 70 °C (at which temperature the spectrum has been completely assigned[5]). A negative sign indicates a downfield shift; spectra were obtained at 270 MHz.

When a solution of vancomycin and Ac-D-Ala-D-Ala (~20 mM in each component) in the above solvent mixture is cooled from 65 to −1 °C, the 1:1 complex goes into slow exchange with its free components. By means of spin-decoupling and variable-temperature studies, it has proved possible to assign the vast majority of proton resonances both in the free components[5,6] and in the complex. The proton resonance shifts which are most important for our arguments are listed in Table I.

The large upfield shift of 208 Hz of methyl resonance α of Ac-D-Ala-D-Ala (**1**) to its position at 0.44 ppm in the complex, which can now be directly observed, confirms earlier conclusions[4-6] that this methyl group lies over an aromatic ring in the complex and that this ring must be the one which is roughly symmetrically located between the two chlorine atoms[1] (Figure 1).

The crucial observation is that resonance H_b (assigned by spin-decoupling) of vancomycin appears at δ 11.7 in the spectrum of the complex, having moved downfield by 3.7 ppm relative to its position in free vancomycin. This large downfield shift is compatible only with the formation of a hydrogen bond between H_b and Ac-D-Ala-D-Ala. In light of the structure of the ristocetin A–Ac-D-Ala-D-Ala complex,[7] there can be no reasonable doubt that the carboxylate anion of Ac-D-Ala-D-Ala is hydrogen bonded to H_{C_4} of vancomycin; this conclusion is supported by the downfield shift (>40 Hz) of H_{C_4} upon complex formation.[5,6] Therefore, vancomycin must undergo a profound conformational change in its "right-hand" portion (relative to the conformation in the crystal, see Figure 2) to permit the formation of a complex in which H_b is near the carboxylate anion of Ac-D-Ala-D-Ala (**1**). The crucial shift of H_b had not been observed in earlier work.[5,6] This is because resonance H_b is lost when the complex is in moderately fast exchange with the free components; under these conditions, it disappears due to exchange broadening.

The proton H_b can only be brought into the proximity of the carboxylate anion of **1** by means of a conformational change involving both the amino acid of which it is part and of the isoasparagine unit. Further evidence that both these amino acids and the *N*-methylleucine are involved in a fundamental conformational change is provided by the large chemical-shift changes undergone by their α-C*H* resonances (v, w, and x, respectively) upon complex formation (Table I). Indeed, the downfield shifts undergone by these three protons upon complex formation are the largest among all those of protons bound to sp^3-hydridized carbon atoms in vancomycin.

The manipulations which must be carried out on the conformation of CDP-I in the crystal (vancomycin is shown in this conformation in Figure 1) to provide a conformation in which both H_b and the terminal *N*-methyl cation can interact with the carboxylate anion of Ac-D-Ala-D-Ala are summarized under Appendix. Although all the manipulations are important and interrelated, the most important to describe is that in which the rigid

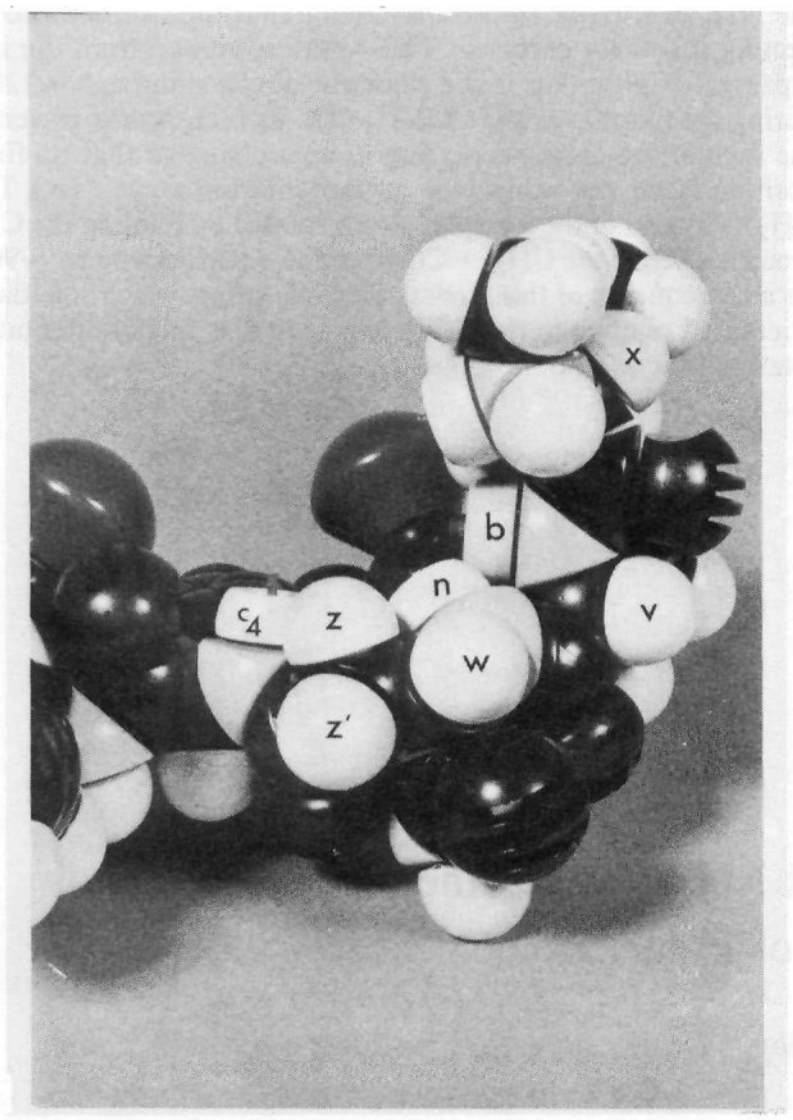


Figure 3. CPK model of the right-hand portion of the structure of vancomycin in the conformation proposed as a binding site for the carboxylate anion of Ac-D-Ala-D-Ala.

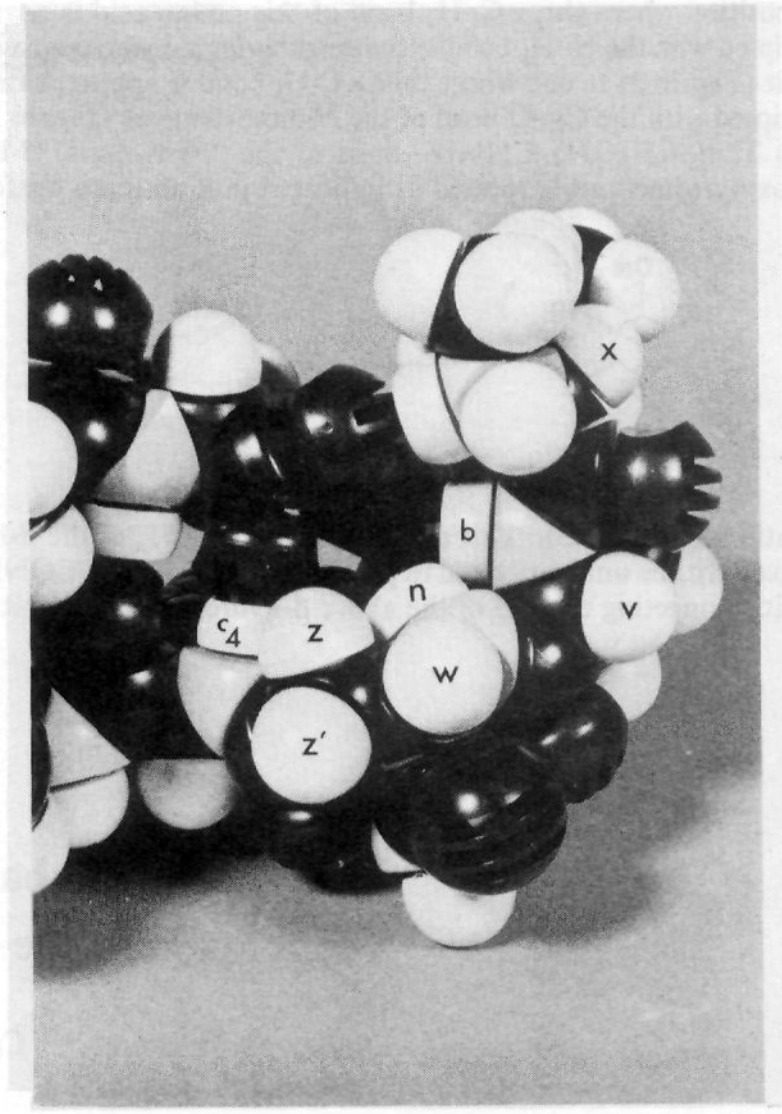


Figure 4. CPK model of the portion of vancomycin shown in Figure 3 with the carboxylate anion of the cell-wall peptide analogue shown in the receptor pocket.

–NHCO– unit bearing NH_n rotates so that H_n moves from the "back face" of the molecule (as conventionally displayed; compare Figures 1 and 2) to the front face (Figure 3). As a consequence, the C=O group of this amide unit moves to the back face and allows H_b to move to a position where it will be able to hydrogen bond to the carboxylate anion (Figure 3). A further change in which the carbonyl group of *N*-methylleucine is maintained in a constant position while the groups attached to the α carbon are rotated by $\sim$180° (by rotation of the α CHCO bond) brings the $NH_2^+CH_3$ into close proximity to the carboxylate anion also. The resulting conformation (Figure 3) allows four NH protons associated with the first four amino acid residues of the antibiotic peptide backbone to interact with the carboxylate anion of the cell-wall analogue. One orientation of the carboxylate anion with respect to these four NH protons is shown in Figure 4. Since hydrogen bonds may involve linear (β-pleated sheets of peptides) or bent (DNA duplex) arrangements of N–H···O=C, Figure 4 is not meant to imply that two linear N–H···O=C interactions shown there are more important than possible nonlinear interactions; it implies only that four interactions (of H_{C_4}, H_n, H_b, and $-NH_2^+-$ protons) to the carboxylate are possible. Indeed, the very large downfield shift (3.7 ppm) of H_b upon complex formation suggests that H_b is involved in formation of the strongest hydrogen bond.

Further evidence in support of our carboxylate anion "receptor pocket" (Figure 4) is available from the data of Convert et al.[6] They noted a marked change in the chemical shifts of the methylene protons H_z and $H_{z'}$ of isoasparagine upon complex formation, and additionally, like Williams and Kalman,[5] they reported a large perturbation (greater than –25 Hz) of the isoasparagine NH_n. Since the NH resonance H_{C_4} also suffers a downfield shift on complex formation (due to hydrogen bonding), it was concluded[6] that the perturbation of H_n could not be due to hydrogen-bond formation since the X-ray structure shows H_{C_4} and H_n to be on opposite sides of the molecule. In the light of our proposed conformational change, it can be seen that the data[5,6] are consistent with the hydrogen bonding of both H_{C_4} and H_n (in addition to H_b) to the carboxylate anion (Figure 4). Finally, we note that in the conformational change (Figure 2 to Figure 3), H_z and $H_{z'}$ do move markedly relative to H_{C_4} (see also Appendix), in accord with their change in chemical shift.

It should of course be considered whether vancomycin, in solution and in the absence of cell-wall analogues, adopts to a significant extent the conformation found for crystalline CDP-I (Figure 1). We conclude that it does in Me_2SO, since in this solvent there is a nuclear Overhauser effect at resonance H_v upon irradiating H_n.[5] This experiment establishes the population of a conformer in which H_n and H_v are in close proximity, which is the case in the conformer found in the crystal (Figure 1) but not that (Figure 3) proposed for the bound state.

The proposed carboxylate anion "receptor pocket" (Figures 3 and 4) bears a remarkable resemblance to that found in the antibiotic ristocetin A.[7,8] However, the receptor pocket of ristocetin A for the carboxylate anion is conformationally rigid, whereas that for vancomycin is formed on demand from a conformationally mobile part of the molecule. Thus, in the dissociation of the vancomycin–Ac-D-Ala-D-Ala complex, numerous rotors will be "unfrozen". This extra loss of order which can occur upon dissociation of the vancomycin–peptide complex may well account for, at least in part, the smaller free energy of activation ($\Delta G^\ddagger$ = 55 kJ mol^{-1}) for dissociation relative to the value ($\Delta G^\ddagger$ $\simeq$ 75 kJ mol^{-1}) found for the ristocetin A–Ac-D-Ala-D-Ala complex.

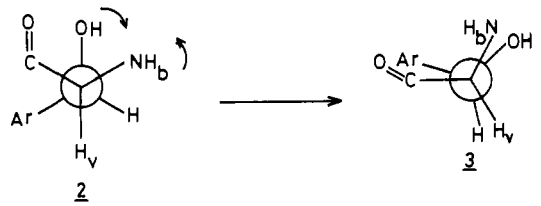
Acknowledgment. We thank the SRC and Churchill College for financial support.

Appendix

Details of the manipulations required to change the conformation of vancomycin from that shown in Figure 2 to that shown in Figure 3 are as follows. (i) The α CH–CO bond of the N-terminal *N*-methylleucine unit is rotated through $\sim$180°: from

(8) Kalman, J. R.; Williams, D. H. *J. Am. Chem. Soc.* **1980**, *102*, 897–905.

a position where the α C-H_x bond of this amino acid is nearly eclipsed with the N-H_b bond of the neighboring β-hydroxytyrosine unit (Figure 2) to one where this α C-H_x bond is approximately eclipsed with the C=O bond of the *N*-methylleucine (Figure 3). (ii) The -CH(OH)-CHNH- bond of the "right-hand" β-hydroxytyrosine unit is rotated as indicated in **2** until the conformation **3** is attained. This change brings the NH_b of the β-hydroxytyrosine unit very close to proton f. (iii) The rigid $CONH_n$ unit, connecting the CO of the above β-hydroxytyrosine unit to the NH_n of isoasparagine, is rotated about the two bonds connecting it to its α carbons. This rotation, viewed from the isoasparagine CH_2 group, is in a clockwise direction through ~120°; during the rotation of the $CONH_n$ unit, its NH_n proton traverses the face of the chlorine-bearing aromatic ring so that its final position is on the same face as the chlorine atom. (iv) The CH_2-CO bond of the isoasparagine is rotated by rotating the CH_2 group, viewed in the $CH_2 \rightarrow CO$ direction, anticlockwise by ~90°. As a consequence of this motion, the CH_2 group, which is initially orientated relative to the CO group as in **4**, is finally orientated relative to it as in **5**.



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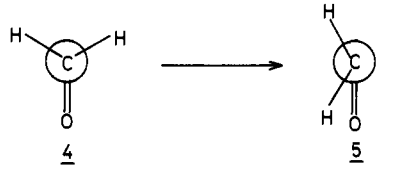

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Fast Atom Bombardment Mass Spectrometry: A Powerful Technique for the Study of Polar Molecules

Dudley H. Williams,*[1a] Carol Bradley,[1a] Gustav Bojesen,[1a] Sitthivet Santikarn,[1a] and Lester C. E. Taylor[1b]

Contribution from the University Chemical Laboratory, Cambridge, United Kingdom, and Kratos, Manchester, United Kingdom. *Received April 3, 1981*

Abstract: A study of a range of polar organic molelcules by fast atom bombardment (FAB) mass spectrometry is reported. Compounds studied include organic salts, polar antibiotics, nucleoside phosphates, and underivatized peptides. In these classes of compounds, molecular weights in the range 300–2000 daltons have been routinely determined, operating in both positive and negative ion modes. Molecular weights of peptides are readily obtained on less than 1 nmol of material, and sequence information is conveniently deduced from sample sizes in the range 2–50 nmol.

In the past, the application of mass spectrometry to the determination of molecular weight and structure of polar molecules has been severely limited. The limitations have arisen due to our inability to produce efficiently the corresponding ions in the gas phase; thermal decomposition of the solid often occurs in preference to volatilization. The advent of field desorption (FD) mass spectrometry[2] has only slightly alleviated the problem; the technique is difficult in practice and the ions providing molecular weight information, if produced at all, are frequently produced only transiently. Californium plasma desorption mass spectrometry[3] has had some spectacular successes, but is not widely available as a routine method. Additionally, secondary ion mass spectrometry (SIMS) has been used to obtain mass spectra of polar organic molecules, but the mass range has so far proved rather limited.[4-6] We now report the application of the technique of fast atom bombardment (FAB), pioneered by Barber and his colleagues,[7] to the study of organic salts, polar antibiotics, nucleoside phosphates, and underivatized peptides. In these classes of compounds, we have routinely determined molecular weights in the range ca. 300–2000 daltons, operating in both positive and negative ion modes.

Experimental Section Mass spectra were obtained on Kratos MS 50 instruments, equipped either with a conventional magnet (mass range ca. 1000 at 8 kV) or an extended range magnet (mass range ca. 1700 at 8 kV). Higher masses were available by operating at lower accelerating voltages. A Kratos commercial FAB source was employed. In this source, argon ions of kinetic energies 4–6 keV are first produced from argon atoms (by electron bombardment and acceleration of the resulting Ar^+ species). The Ar^+ ions of high translational energy (ion beam current ca 40 μamps) are then converted to Ar atoms of similar energy by charge exchange on passing through argon gas. The argon gun does not employ an electric sector to remove any residual ions which may exist in the beam of Ar atoms. The 4–6 keV beam of atoms is then impacted onto the sample. We have normally dissolved, or dispersed, the sample in glycerol; such a matrix facilitates the production of sample ions in high abundance for relatively long periods. The solution, or suspension, is then introduced into the source on a copper probe tip. The area of the sample matrix on

* Address correspondence to this author at the University Chemical Laboratory.

(1) (a) University Chemical Laboratory, Lensfield Road, Cambridge. (b) Kratos, Ltd., Barton Dock Road, Urmston, Manchester.
(2) Beckey, H. Jd. "Principles of Field Ionisation and Field Desorption Mass Spectrometry"; Pergamon: New York, 1978. For the use of FD emitters in chemical ionization plasma, see: Hunt, D. F.; Shabanowitz, J.; Botz, F. K.; Brent, D. A. *Anal. Chem.* **1977**, *49*, 1160.
(3) MacFarlane, R. D.; Torgerson, D. F. *Science* **1976**, *191*, 920.
(4) Benninghoven, A.; Sichtermann, W. *Org. Mass Spectrom.* **1977**, *12*, 595.
(5) Grade, H.; Winograd, N; Cooks, R. G. *J. Am. Chem. Soc.* **1977**, *99*, 7725. Day, R. J.; Unger, S. E.; Cooks, R. G. *Ibid.* **1979**, *101*, 501.
(6) Kambara, H; Hishida, S. *Org. Mass Spectrom.* **1981**, *16*, 167.
(7) Barber, M.; Bordoli, R. S.; Sedgwick, R. D.; Tyler, A. N. *J. Chem. Soc., Chem. Commun.* **1981**, 325. See also: Surman, D. J.; Vickerman, J. C. *Ibid.* **1981**, 324.

0002-7863/81/1503-5700$01.25/0