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THE ^{13}C MAGNETIC RESONANCE SPECTRUM OF THE ANTIBIOTIC VANCOMYCIN

Dudley H. Williams and John R. Kalman

University Chemical Laboratory, Lensfield Road,

Cambridge CB2 1EW, U.K.

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Vancomycin [1] is a glycopeptide antibiotic used clinically in resistant and severe cases of infection by Gram-positive micro-organisms. In the 20 year period during which vancomycin has been known, a direct determination of its molecular weight (≃ 1500 daltons) by mass spectrometry has proved impossible due to the involatility and thermal instability of the antibiotic and its derivatives. By means of chemical degradation experiments [2,3] and a proton nmr study at 270 MHz [4] we have concluded that the molecular formula is $C_{66}H_{75}Cl_2N_9O_{24}$, and that the molecule incorporates six secondary amides, one primary amide, and a carboxylic acid (eight C=O groups in all). It was desirable to confirm these conclusions by ^{13}C magnetic resonance, but earlier experiments [3] at 25.1 MHz gave spectra with overlapping signals that could not be analysed. We now report a study at 67.7 MHz, from which it is concluded that vancomycin does indeed contain 66±1 carbons atoms, of which 8 are carbonyl carbons.

The ^{13}C spectrum of vancomycin hydrochloride (200 mg) in $\underline{d}_6$-DMSO (1.5 ml)[5] shows the resonances listed in the Table, in particular, eight in the range 166-173 ppm due to carbonyl groups. An additional spectrum, recorded in D_2O solvent[5], establishes that two carbon resonances are hidden under that of DMSO in the previous experiment. The only ambiguity remaining in the original spectrum is the number of carbons associated with a broad peak at 127 ppm. This peak, when expanded, shows two resolved maxima and two inflections, and based on the average intensity of the resolved resonances in this region, corresponds to 4±1 carbons. Our proposals [2-4] for the structure 1 incorporate five benzene rings and the anomeric carbons of glucose and vancosamine, requiring [6] 32 resonances in the region 96-158 ppm, in accord with 32±1 found in the present study.

The proposed structure 1 also contains eight aromatic carbons bound to oxygen. The assignment of seven of these to the resonances in the region 148-158 ppm is supported by the narrowness of the lines, which indicates the absence of directly bonded hydrogens. The oxygen-bearing aromatic carbon which lies outside this range should be, on the basis of chemical shift considerations, C-2 in the 1,2,3-trioxygenated aromatic ring. This carbon should suffer a large upfield shift due to the presence of two ortho substituents[6], and its chemical shift is thus estimated to lie in the region 125-140 ppm.

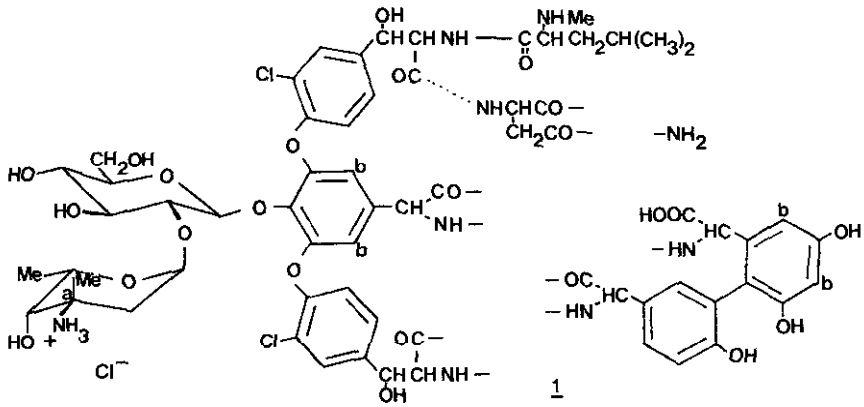

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Table: Summary of ^{13}C Nmr spectrum of Vancomycin in d_6-DMSO

chemical shift range (ppm) ex TMS	number of carbons	assignments [6]
0 - 40	9	8 sp^3 carbons not attached to an electron-negative atom; $N-CH_3$ resonance.
50 - 60	10	7 amino acid α-CH; CH_2OH of glucose; C-3 in vancosamine (marked 'a' in 1); one sp^3 carbon bearing a single oxygen.
70 - 80	7	7 sp^3 carbons bearing a single oxygen.
95 - 110	6	2 anomeric sugar carbons; 4 aromatic carbons with two hydroxyl groups in ortho or para positions (marked 'b' in 1).
115 - 145	19 ± 1	19±1 aromatic carbons.
148 - 158	7	7 aromatic carbons bearing an oxygen substituent.
165 - 175	8	8 C=O groups.

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Footnote and References

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2. K. A. Smith, D. H. Williams, and G. A. Smith, J. Chem. Soc. Perkin I, 1974, 2369.

3. G. A. Smith, K. A. Smith, and D. H. Williams, J. Chem. Soc. Perkin I, 1975, 2108.

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5. The spectrum was recorded in the Fourier Transform mode (40,000 transients accumulated) using a 45° pulse, proton noise decoupling, and an acquisition time of 0.94 sec. without pulse delay.

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