## Concerning the Molecular Weight and Structure of the Antibiotic Vancomycin

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Summary The known fragments of the antibiotic vancomycin have been extended to account for almost 75% of the molecular weight of the antibiotic. VANCOMYCIN is an antibiotic which has been used in the treatment of staphylococcal infections.<sup>1</sup> Interest in its structure has recently been aroused by the discovery that it complexes selectively with peptides having the C-

terminal sequence D-Ala-D-Ala.<sup>2,3</sup> This is probably the basis of its antibiotic activity.<sup>2</sup> A molecular weight of 3000 was first put forward from sedimentation data,<sup>4</sup> but values of 1600,<sup>5</sup> and 1700-1800 have been suggested more recently.6

The degradation product, CDP-(I), is produced from vancomycin, with the loss of ammonia, in yields of up to 93% by weight.<sup>7</sup> It is therefore likely that the molecular weights of CDP-(I) and vancomycin are similar. A previous, unpublished, X-ray study gave a molecular weight of 1840 for CDP-(I).7

In an attempt to examine the structure of CDP-(I) by X-ray diffraction, preliminary photographic results have been obtained. CDP-(I) crystallizes in space group  $P2_{1}2_{1}2_{1}$  with a = 13.90(3), b = 21.13(5), c = 33.09(4) Å,  $D_{\rm m} = 1.326(5) \text{ g cm}^{-3}$  (by density gradient column). With this value of the density and assuming four molecules per unit cell, the formula weight was calculated to be 1940 + 17.

Since the crystals disintegrate under atmospheric conditions, our study was conducted with the crystal immersed in its mother liquor in a capillary. Despite this precaution, however, no diffraction pattern could be observed above  $2\theta = 60^{\circ}$  (Cu- $K_{\alpha}$ ,  $\lambda = 1.5418$  Å).

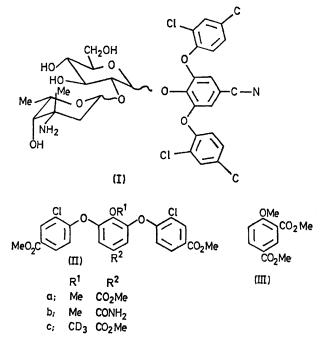
The water content of the crystals was measured by observing the weight loss of single crystals suspended over phosphorus pentoxide. The weight became constant within 48 h and the loss observed was 27.4 (+1.0)%. This indicates an anhydrous molecular weight of about 1420. Analysis of this dried material gave a formula of C<sub>64</sub>H<sub>74</sub>N<sub>8</sub>- $O_{26}Cl_2$  (M.W. = 1441).

With approximately 28 water molecules per asymmetric unit the structure is likely to be disordered, and further X-ray work on the crystals is not practicable. We hope, however, to prepare a complex of CDP-(I), more suitable for X-ray diffraction analysis, and continue the structural investigation.

Concurrent with the X-ray work, chemical studies were carried out leading to the partial structure, (I), for vancomycin. The configuration of the glycoside bonds in the suggested partial structure is not yet established.

Vancomycin was permethylated (dimethyl sulphoxide anion, followed by an excess of methyl iodide), and the product hydrolysed (MeOH-HCl). A portion of the resulting partially methylated glucose was further methylated (-CH<sub>2</sub>SOMe-CD<sub>3</sub>I), and the mass spectrum of the product established the position of deuteriomethylation as C-2.8 Alternatively, the partially methylated glucose was reduced (NaBH<sub>4</sub>) and acetylated (Ac<sub>2</sub>O-py) to give 1,2,5-tri-Oacetyl-3,4,6-tri-O-methylhexitol, which was identified from its characteristic mass spectrum (determined by the direct insertion method).<sup>9</sup> These experiments established that the recently isolated sugar, vancosamine,<sup>10,11</sup> is linked to glucose at position 2, thus confirming the tentative conclusion of Johnson, Smith, and Guthrie.11

The aromatic portion of (I) was established via the



following sequence of reactions. Aglucovancomycin7 was methylated (MeI-K<sub>2</sub>CO<sub>3</sub>-MeOH) and the product oxidised with aqueous permanganate at pH 8 at 75° for 3 h. The ethyl acetate soluble products were methylated (CH<sub>2</sub>N<sub>2</sub>) and (IIa) and (IIb) identified from microanalytical and spectroscopic data. The further product, (III), isolated from this reaction, provides evidence for another aromatic ring in vancomycin.

The attachment of the sugars to the aromatic rings was established by the following sequence of reactions: (1) methylation of vancomycin (MeI-K<sub>2</sub>CO<sub>3</sub>-MeOH), (2) removal of the sugars by hydrolysis (HCl-MeOH), (3) trideuteriomethylation (CD<sub>3</sub>I-K<sub>2</sub>CO<sub>3</sub>-MeOH), (4) oxidation (permanganate) and methylation  $(CH_2N_2)$  as described above. This sequence gave (IIc).

Electrophoresis of vancomycin and of some of its derivatives indicates that vancomycin contains one carboxygroup and two amino-groups, consistent with recent titration data.<sup>6</sup> Furthermore, these experiments indicate that CDP-(I) contains two carboxy-groups, suggesting a primary amide group in vancomycin.

The known fragments of vancomycin, *i.e.* (I), the methylated product (III), N-methyl-leucine, and aspartic acid,<sup>7</sup> now account for a molecular weight of 1040, *i.e.* almost 75% of the molecular weight of CDP-(I) above.

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