

CHEMISTRY OF BLEOMYCIN. XX
THE X-RAY STRUCTURE DETERMINA-
TION OF P-3A Cu(II)-COMPLEX,
A BIOSYNTHETIC INTERMEDIATE
OF BLEOMYCIN

Sir:

During a biosynthetic study of bleomycin (BLM), several peptides, structurally related to BLM, were isolated from the culture broth of *Streptomyces verticillus*. One of them, designated as P-3A, was crystallized from water (NAKATANI *et al.*, unpublished) as an equimolar Cu(II)-complex. Acid hydrolysis of metal-free P-3A gave β -aminoalanine (amine component V¹) of BLM), 2-(1-amino-2-carboxyethyl)-6-aminopyrimidine-4-carboxylic acid (a demethyl analogue of amine component II²) of BLM, demethyl-II), L-histidine and L-alanine. Terminal group analysis of P-3A showed that the α -amino group of V and the carboxyl group of alanine are free by SANGER'S DNP-method and by AKABORI'S hydrazinolysis method, respectively. The ¹³C-NMR spectrum of P-3A³) indicated that it consists of one mole each of the above 4 amino acids. However, the molecular formula of P-3A could not be established definitely from the elemental analysis, P-3A Cu(II)-complex, Found: C, 39.43; H, 5.08; N, 24.26; Cu, 10.37. Metal-free P-3A, Found: C, 43.52; H, 6.02; N, 26.92. In this communication, the structure of P-3A Cu(II)-complex as determined by X-ray crystallographic analysis is presented.

The crystals of P-3A Cu(II)-complex as recrystallized from aqueous solutions were rhombohedral in shape and dark blue in color. As the crystals readily deteriorated in air by losing water of crystallization, they were sealed in glass capillaries prior to exposure to X-rays. A specimen of the approximate dimensions 0.2 × 0.2 × 0.25 mm was mounted on a Philips PW-1100 diffractometer and the unit cell dimensions and intensity data were measured with monochromatic (graphite plate) CuK α radiation. The cell dimensions a=b=17.075(9), c=21.289(11) Å, γ =120°, and the symmetry of the intensity distribution indicated that the LAUE group of the crystal must be $\bar{3}m$. The space group was at first assumed to be P3₁21* taking

* The possibility of the alternative space group P3₂21 has been eliminated by assuming the absolute configurations of L-histidine and L-alanine residues.

into account the LAUE group and the absent reflexions, $l=3$ in 0 0 l . The density of the crystal measured by the flotation method using benzene—carbontetrachloride solutions was 1.47 gcm⁻³ which showed that the molecular weight for a structure unit in an asymmetric unit is 793 assuming that 6 units are contained in a cell. Although the molecular weight was not known exactly, the most probable formula derived from the elemental analysis was C₂₀H₂₇N₁₁O₆Cu·2H₂O, which gave the molecular weight 581 and the calculated weight compositions of C, 38.93; H, 5.06; N, 24.97; O, 20.74; Cu, 10.30. The difference between 793 and 581 should be due to additional water of crystallization contained in an asymmetric unit, corresponding to 11.8 molecules of water for every one molecule of P-3A Cu-complex.

Intensities for about 5100 reflexions, including many crystallographically equivalent reflexions, were measured within a 2 θ range of 0~120° and a total of 1817 hk·l and 1103 $\bar{h}\bar{k}\cdot\bar{l}$ structure factors were obtained.

The crystal structure was solved by the heavy atom method and the entire structure was revealed by the repeated cycles of structure factor—FOURIER and least-squares calculations. Refinement by the block-diagonal least-squares method including anisotropic temperature factors gave an R factor of 0.153 for all the 38 atoms of P-3A Cu-complex and 4 water oxygen atoms. However, the electron density map showed some unusual features for two terminal groups. The C-terminal carboxylic acid group of L-alanine and the "carboxylic acid or carboxamide" side-chain of demethyl-II appeared to exhibit unusually large thermal vibrations. The vibrations of the former group could be explained by vibrational motion about the C-C' (carboxyl carbon atom) bond whereas those of the latter group could not be interpreted by such a simple rotational motion. Furthermore, the intermolecular distances between the molecules related by a diad axis exhibited some unusually short contacts between these demethyl-II side-chains.

It was therefore assumed that the symmetry of the space group P3₁21 was violated in this part of the molecule although no definite deviation of the intensity distribution from that expected for the point group 32 symmetry had been observed. Refinement of the structure in the space group P3₁ was then carried out including the

76 non-hydrogen atoms of the two complex molecules and 12 oxygen atoms from water molecules. The difference electron density map calculated by excluding the above mentioned side-chain atoms could be interpreted in terms of a disordered model for the side-chain (2-carboxy or carboxamide group of demethyl-II). This model assumes two alternative positions for the side-chain with different rotations about the ethyl C-C bond as well as those about the C(ethyl)-C' bond. The refinement was continued by estimating occupancy factors of the atoms for each rotational state to be 0.5 on the basis of the mean peak height for the atoms on the difference electron-density map. These occupancy factors and positional parameters of the disordered side-chain atoms (the atomic species were not identified for these atoms and they were all assumed to be carbon atoms) were fixed in the least-squares calculations, since the correlations between these parameters seemed to be

Fig. 1. (a) A perspective drawing of the molecule B of P-3A. Atoms are shown by ellipsoids of 10% probability. Side chain atoms of demethyl-II, (11), (12) and (13) are disordered and their alternative positions are also drawn. The assignment of atomic species for these atoms was not made by X-ray methods.

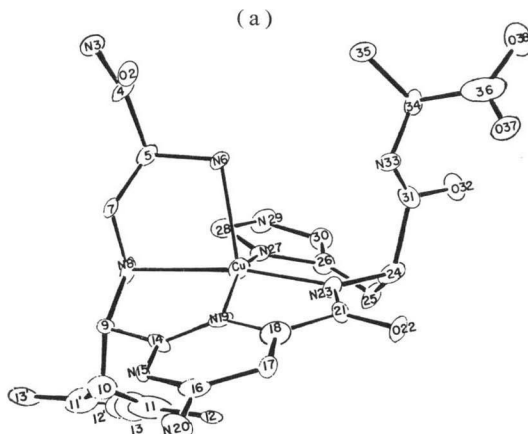
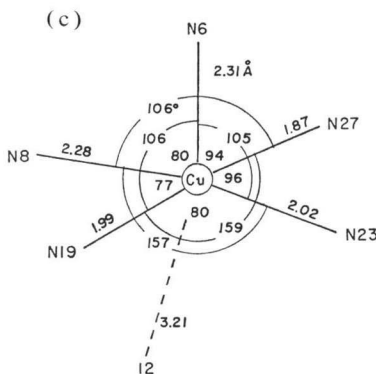
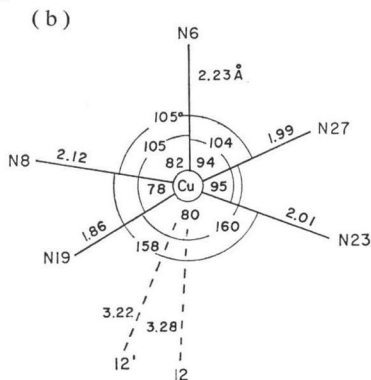
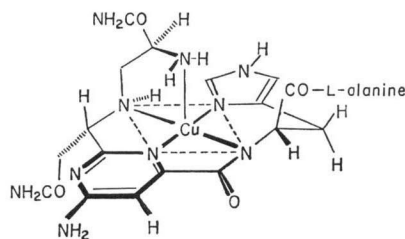


Fig. 1. (b) Coordination about the copper ion in molecule A and (c) in molecule B. Bond lengths and angles are shown in Å unit and in degrees and their standard deviations are estimated to be ± 0.03 Å and $\pm 1^\circ$, respectively. The copper ion is coordinated to the 5 nitrogen atoms as shown by solid lines. The distance to the atom (one of the terminal atoms of the carboxamide group of demethyl-II) situated at the position in proximity to that corresponding to the 6th ligand site is also indicated.



very large. The R factor was reduced to 0.131 for 3022 reflexions taking into account the contributions of 84 non-hydrogen atoms of the two complex molecules (including 8 alternative positions for the side-chain atoms) and 15 water oxygen atoms. Further refinement by the least-squares calculations along the same line did not improve the R factor. This may be due to the disorders of the side-chain atoms and water molecules. The results obtained at this stage are

Fig. 2. The structure of Cu(II)-complex of P-3A



therefore presented below.* Full details will be published later.

The structure of one of the molecules, (B), in the asymmetric unit is shown in Fig. 1 by an ORTEP drawing. The constitution of the molecule (Fig. 2) consists of an amine component V, demethyl-II, L-histidine and L-alanine linked together through the amide or C-N-C bonds.

The Cu ion is bound to five nitrogen atoms, which complete a square pyramidal coordination. These nitrogen atoms are: the secondary amino nitrogen linking V and demethyl-II, the ring nitrogen-1 of the 4-aminopyrimidine nucleus of demethyl-II, the deprotonated amide nitrogen of L-His, the N^π of the imidazole of L-His and the α-amino nitrogen atom of V. The basal plane of the coordination is formed by the first 4 of these nitrogen atoms; Cu-N distances ranging from 1.86 to 2.12 Å in molecule A and from 1.87 to 2.28 Å in molecule B. In both molecules, the Cu ion is displaced about 0.20 Å from the basal plane in the direction of the 5th ligand nitrogen atom of the α-amino group of V. The distance to the 5th ligand atom is 2.23 Å in A and 2.31 Å in molecule B. As described above, the side-chain of demethyl-II adopts a disordered conformation. The final difference electron-density map also showed additional rotation for the side-chain of molecule A but the electron-densities were diffuse and quite small and so this disorder was not taken into account in the refinement. In Fig. 1, various conformations of the side-chain group are superposed.

It must be noted that, due to the disorder, it was not possible to determine uniquely the structure of the terminal group of the side-chain. If the structure of this part is present as a carboxylic acid, the N/C molar ratio of P-3A is 10/20, and if it is a carboxamide, the ratio is 11/20. But the N/C ratio found from the elemental analysis was 10.56/20 and 10.62/20 for the Cu-complex and the metal-free P-3A, respectively. The structure of this group was finally determined to be a carboxamide by comparison of the ¹³C-NMR spectra of copper-free P-3A and pseudodipeptide triamide³¹. That is, the ¹³C-NMR chemical shifts of the 6 carbon atoms at

* A list of observed and calculated structure factors, positional and anisotropic thermal parameters at the present stage of refinement will be obtained from the first named author upon request.

the pyrimidine ring-2 substituent of both compounds were almost the same.

The present X-ray crystallographic study has elucidated the structure of the P-3A Cu(II)-complex which will contribute to the studies on the conclusive structure determination of BLM and also to clarify the nature of the coordination in the BLM metal-complex, a factor which should play an important role in the bioactivity of BLM. The paper describing the structure of BLM³¹ has been published and that describing the structure of BLM metal-complex⁴¹ will be published soon.

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