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THE CHEMISTRY OF BLEOMYCIN. II THE MOLECULAR AND CRYSTAL STRUCTURE OF A SULFUR-CONTAINING CHROMOPHORIC AMINO ACID

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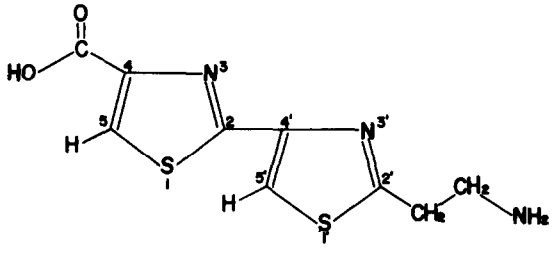
As reported in previous papers, bleomycins[1,2] and phleomycins[3,4,5] have a similar chromophore which is characterized by an ultraviolet absorption maximum near at 290 mμ. A compound (I) $C_9H_9N_3O_2S_2 \cdot H_2O$ corresponding to the chromophore was isolated by the acid hydrolysis of bleomycin A_2. (I) shows the u.v. absorption maximum at 290 mμ ($\log \varepsilon$ =4.1 in 0.1N HCl) and no other fragment having u.v. absorption maxima near at 290 mμ is obtained by the degradation of bleomycins. In the present paper, the authors report the structure of this compound determined by X-ray analysis of its methyl ester monohydrobromide as follows:



2'-(2-aminoethyl)-2,4'-bithiazole-4-carboxylic acid

Crystals of monohydrobromide of methyl ester of (I) are colorless prisms which contain one molecule of crystal water; the analytical data coincide with the formula of $C_{10}H_{11}N_3O_2S_2 \cdot HBr \cdot H_2O$, m.w. 368.3 (373 by X-ray method); m.p. 233-233.5°C. The crystal belongs to triclinic system, space group $P\bar{1}$ with two structure units in the unit cell of dimensions, a=11.08Å, b=10.97Å, c=7.34Å, α=89.2°, β=107.5° and γ=120.1° which were determined from the precession photographs of hk0, h0ℓ and 0kℓ taken with CuK_α radiation (λ=1.5418Å). The density measured by the flotation method using a mixed solution of carbon tetrachloride and bromoform is 1.659 gr/cm^3 which coincides with the calculated value of 1.637 gr/cm^3. Three-dimensional intensity data were collected from the equi-inclination Weissenberg photographs around the c-axis. The layers from hk0 up to hk4 were taken by CuK_α radiation using the multiple film technique. The intensities were estimated visually by the use of the calibrated intensity scale.

The analysis was first carried out on the c-axis projection. A two-dimensional Patterson function was calculated on 216 observed structure factors and the position of a bromine atom was determined. A two-dimensional Fourier synthesis using the phases of the Fourier coefficients based upon the contribution of the bromine atom was computed and two sulfur atoms could be recognized in the resulting electron density map (R=46.5%). Several subsequent calculations of Fourier and difference Fourier syntheses revealed the whole structure together with a molecule of crystal water. This structure was refined by the least-squares method (R=16.9%). A three-dimensional Patterson synthesis was then calculated and it was tried to obtain the z-coordinate of each atom satisfying the results of the two-dimensional analysis. As a result, z-coordinates of all the atoms in the asymmetric unit, i.e. a bromine, two sulfurs, three oxygens, three nitrogens and ten carbons could be obtained by the three-dimensional Patterson synthesis very reasonably. The atomic parameters of this structure were then subjected to seven cycles of the least-squares refinement which reduced the reliability factor from 27.4% to 16.8%. The calculations of four more cycles of the least-squares refinement in which the anisotropic thermal parameters for each atom were applied gave the reliability factor of 10.3% for 1777 reflections. The composite electron density map obtained by the final calculation of Fourier synthesis is shown in Fig. 1.

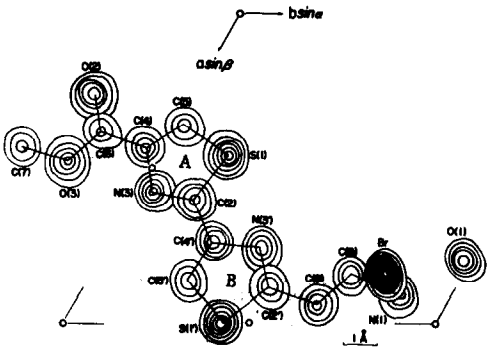


FIG. 1 Composite Electron Density Map

Both of the two thiazole rings, A and B, are completely planar, the dihedral angle between these two planes being 6°. The side chain moieties also, especially the carbonyl group on the A ring, are in the plane of the thiazole rings. 2-Aminoethyl residue takes the planar zig-zag conformation nearly in plane of B ring. Consequently, the molecule has a nearly planar structure as a whole. The intramolecular bond distances and angles are shown in Fig. 2. The average standard deviations of the bond distances and angles are 0.02Å and 1.7°. The present study is the first case of the crystal structure analysis of the molecule which contains a bithiazole ring. The chemical structure of (I) has not been found in substances of the natural source. In the biogenetical consideration, the structure of (I) is quite reasonable, being composed of two molecules of cysteine and one molecule of β-alanine.

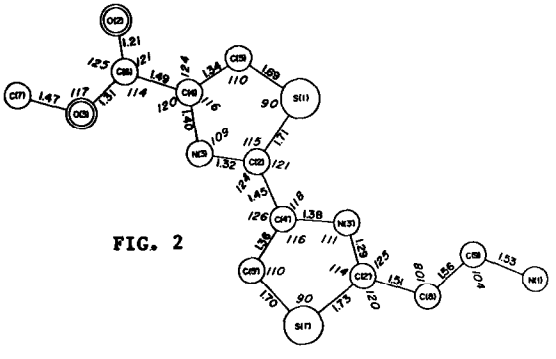


FIG. 2

The calculations in the present study were performed on the HITAC 5020E computor at the University of Tokyo and CDC 3600 computor at the JAIF Control Data Centre, Tokyo. A full description of the present structure determination will be published later.

REFERENCES

1. H. Umezawa, K. Maeda, T. Takeuchi and Y. Okami, J. Antibiotics, 19A, 200 (1966).
2. H. Umezawa, Y. Suhara, T. Takita and K. Maeda, J. Antibiotics, 19A, 210 (1966).
3. K. Maeda, H. Kosaka, K. Yagishita and H. Umezawa, J. Antibiotics, 9A, 82 (1956).
4. T. Takita, K. Maeda and H. Umezawa, J. Antibiotics, 12A, 111 (1959).
5. T. Takita, J. Antibiotics, 12A, 285 (1959).