PIPERAZINOMYCIN, A NEW ANTIFUNGAL ANTIBIOTIC

II. STRUCTURE DETERMINATION BY X-RAY CRYSTALLOGRAPHY

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The structure and absolute configuration of piperazinomycin have been established by X-ray crystallographic analysis of its monohydrobromide.

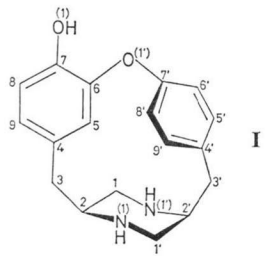
In the preceding paper,[1] the fermentation, isolation, characterization and biological properties of piperazinomycin were reported. In this paper, we wish to describe the X-ray crystallographic analysis of piperazinomycin monohydrobromide which results in the establishment of the chemical structure and the absolute configuration of piperazinomycin (**I**) (Fig. 1).

Piperazinomycin monohydrobromide (**II**) was prepared by treating **I** in methanol with an equimolar amount of hydrogen bromide in methanol. Light yellow prisms of **II** were grown from the methanol solution by the vapor diffusion method in which the test tube containing the methanol solution of **II** was allowed to stand open in a sealed flask containing acetone.

Preliminary X-ray photographs showed that the crystal belonged to the orthorhombic system. The size of the crystal used for the data collection was about $0.6\times0.4\times0.15$ mm The lattice constants and intensity data were obtained on a Syntex R3 computer-controlled four circle diffractometer using MoKα radiation monochromated by a graphite plate. The crystal data are given in Table 1. A total of 2304 reflections was measured by the $\theta-2\theta$ scan method within a 2θ range of $0\sim50°$; of these 2047 reflections including 847 Friedel pairs with intensities above 1.96σ(Io) level were used in the structure determination. During the data collection, two reference reflections were monitored every 100 reflections and no significant drop in the intensity was observed. The intensities were corrected for Lorentz and polarization factors, but not for absorption.

The structure was solved by the heavy atom method. All non-hydrogen atoms were located by

Fig. 1. Chemical structure and absolute configuration of piperazinomycin (**I**).



I

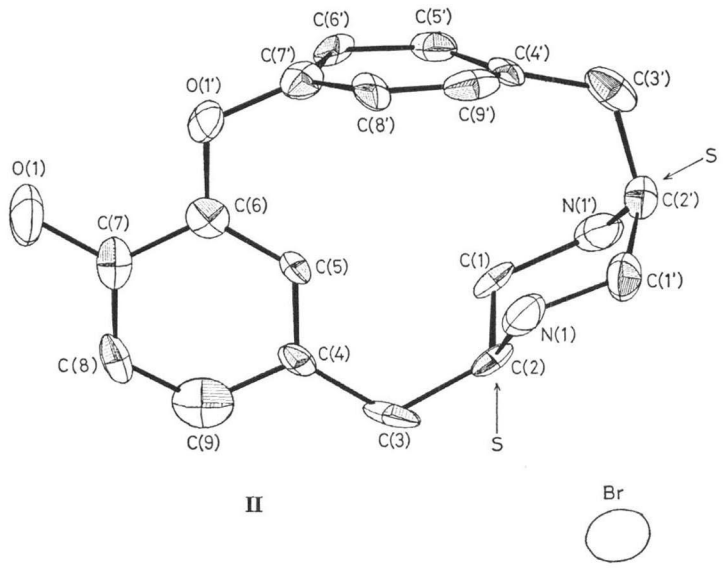
Table 1. Crystal data of piperazinomycin monohydrobromide (**II**).

Piperazinomycin monohydrobromide, $C_{18}H_{20}N_2O_2\cdot HBr$, MW=377.1
Orthorhombic, space group $P2_12_12_1$
a=8.926(5), b=22.341(11), c=8.159(2) Å, Z=4,
Dc=1.540 g/cm^3, U=1627.0 Å^3.

successive use of difference Fourier synthesis and were refined by least-squares methods. The atomic species were assigned on the basis of the Fourier map and the temperature factors with the help of chemical considerations. Further refinement by block-diagonal least-squares methods with anisotropic temperature factors for all non-hydrogen atoms reduced the R factor to 0.096. The absolute configuration was determined by the anomalous dispersion method. The dispersion terms of the bromine atom for MoKα radiation were assumed to be $f'=-0.374$ and $f''=2.456$, and were taken into account in the structure factor calculations. The observed and calculated BIJVOET inequalities[2] for the reflections with values of $||Fobs(hkl)|-|Fobs(\bar{h}\bar{k}\bar{l})||>3\sigma|Fobs(hkl)|$ were examined and 235 out of 281 Friedel pairs were in good agreement with each other. Therefore, the absolute configuration corresponds to a right-handed axial system for the atomic coordinates. Finally, the anomalous dispersion effect was taken into account in the least-squares refinements.[3] The R factor calculated for the absolute configuration derived above was 0.083 and that for the enantiomer was 0.101. These results established the structure of piperazinomycin monohydrobromide including the absolute configuration to be **II** as shown in the ORTEP drawing[4] of Fig. 2. The bond lengths and angles agree quite well with the chemical structure. Thus, the chemical structure and the absolute configuration of piperazinomycin (**I**) have unambiguously been established as shown in Fig. 1, the chiral centers, C(2) and C(2′), both being *S*.

Piperazinomycin (**I**) has a unique cyclic structure in which two benzene rings and one piperazine ring are linked together by three separate atoms, O(1′), C(3) and C(3′). The piperazine ring is in an almost ideal chair conformation with C(3) and C(3′) deviating from the mean plane of the piperazine ring by 0.06Å (equatorial) and 1.58Å (axial), respectively. The benzene ring (ring A) formed by C(4), C(5), C(6), C(7), C(8) and C(9) is quite planar with an average deviation of $\pm$0.02Å, but the other benzene ring (ring B) formed by C(4′)~(9′) is deformed with an average deviation of $\pm$0.05Å with C(4′) and C(7′) deviating from the best plane of the ring in the same direction by 0.08Å and 0.07Å,

Fig. 2. Perspective drawing of the molecule of piperazinomycin monohydrobromide (**II**) showing the correct absolute configuration.



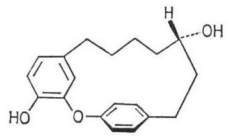
II

respectively. The B ring is pulled through the bonds, C(4′)–C(3′) and C(7′)–O(1′), towards the piperazine ring and benzene ring A. In fact, C(3′) and O(1′) deviate from the best plane of benzene ring B by 0.40Å and 0.58Å, respectively. The dihedral angle between the best planes of the two benzene rings is 80.4°, that between benzene ring A and the piperazine ring is 86.7°, and that between benzene ring B and the piperazine ring is 39.5°. The hydroxyl group O(1)H forms an intramolecular hydrogen bond to O(1′) of the diphenyl ether [O(1)–O(1′), 2.726(10)Å]. The bromide anion also forms the following three hydrogen bonds [Br at x, y, z—O(1) at x, y, z, 3.279(8)Å; Br at x, y, z—N(1′) at x, y, z, 3.317(8)Å; Br at x, y, z—N(1′) at $-x$, $1/2+y$, $-1/2-z$, 3.296(9)Å]. These hydrogen bonds involving the bromide anion hold the molecules in the crystal to form a three-dimensional network.

The rotation of benzene ring B along the C(4′)–C(7′) axis seems to be hindered and this hindrance may be maintained even in solution as the proton signals for benzene ring B in the ^{1}H NMR spectrum of **I** did not exhibit a simple A_2B_2 type coupling pattern but a rather complicated splitting pattern due to inequality between C(6′)–H and C(8′)–H, and C(5′)–H and C(9′)–H. Furthermore, since the two benzene ring planes are nearly perpendicular to each other, the hydrogen atom at C(5) lies just over benzene ring B. Accordingly, the proton at C(5) may be strongly shielded by the ring current to give a signal at a remarkably higher field in the ^{1}H NMR spectrum than ordinary aromatic protons. Therefore, the doublet proton signal at δ 5.76 can be assigned to the C(5) proton, and consequently, the proton signals at δ 6.65 and 6.40 can be attributed to C(8)–H and C(9)–H, respectively.[1] A similar steric situation for a hydrogen atom was previously reported for acerogenin A (**III**)[5,6] (Fig. 3) which was isolated as the aglycone of a glucoside from the stem bark of *Acer nikoense* Maxim. (Aceraceae) and has the identical aromatic portion in its large cyclic structure. In the case of acerogenin A, the corresponding proton signal (6-H) was observed at δ 5.84 (1H, d, J=2 Hz) in C_5D_5N.

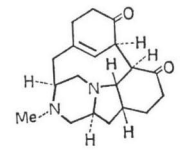
Biogenetically, it is assumed that piperazinomycin (**I**) may be made up from two molecules of tyrosine. From this viewpoint, it is interesting that the absolute configurations of C(2) and C(2′) of piperazinomycin (**I**) correspond to that for C(2) of L-tyrosine. A biogenetically related fungal metabolite, herquline (**IV**), was reported and was also assumed to be derived from two molecules of tyrosine[7,8] (Fig. 4).

Fig. 3. Structure of acerogenin A.



III

Fig. 4. Structure of herquline.



IV

Acknowledgment

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