The Structure of Bromoemimycin

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The purpose of this investigation was to elucidate the molecular structure of emimycin by means of X-ray crystal analysis. Emimycin is an antibiotic which is obtained from Streptomyces griseochromogenes.¹⁾ It has a partial inhibition against various species of bacteria and some yeasts. The chemical formula $C_8H_8N_4O_4$ was assigned to emimycin on the basis of the chemical analysis and molecular weight determination. When we started our crystal structure analysis of emimycin, nothing was known of its structure except that it had one hydroxyl group. By the direct bromination of emimycin with aqueous bromine, a bromine derivative was obtained in a form of crystals suitable for X-ray analysis. To this compound as used in this investigation, a compound which we call bromoemimycin, the formula $C_8H_6N_4Br_2O_4$ was assigned.

Experimental

Crystals of bromoemimycin were obtained by letting an aqueous solution stand for a week in a refrigerator. They are colorless long needles elongated along the c-axis. The crystal was found to be orthorhombic. The dimensions of the unit cell were obtained using higher order reflections on oscillation photographs:

$$a = 8.68\pm0.03\,\text{Å}$$
$$b = 11.84\pm0.05\,\text{Å}$$
$$c = 5.35\pm0.02\,\text{Å}$$
$$\text{Volume} = 549\,\text{Å}^3$$

The systematic absences were found to be $(0\,k\,l)$ for $k+l$ odd, and $(h\,0\,l)$ for h odd. Therefore, the space group was determined to be $Pna2_1$ or Pnam. These space groups require four or eight molecules in the unit cell. The density, as measured by the floatation method using a mixture of carbon tetrachloride and tetrabromoethane, was 2.25 g.cm^{-3}. From the volume of the unit cell and the density of the crystal thus obtained, the molecular weights are calculated to be 186 or 93, which are approximately one-half or one-quarter of the expected value. However, the latter value is unlikely, because the total atomic weights of the chemical formula requires 191 as the least value. This suggests that the true moleular formula should be $C_4H_3N_2BrO_2$, which gives a calculated density of 2.31 g.cm^{-3}, compatible with the observed value.

Intensities were obtained by the multiple-film technique, using an integrated Weissenberg camera. Filtered CuK_α radiation was used throughout. Three-dimensional intensity data were collected for $(h\,k\,l)$ l up to 3, for $(h\,k\,l)$ h up to 3, and for $(h\,0\,l)$ and $(h\,1\,l)$. The intensities were visually estimated using a standard calibrated strip.

Correction for the Lorentz and polarization factors were made in the usual way. Absorption correction was not made, since the cross-sections of the specimens used are 0.03×0.05 mm. for the c-axis and 0.02×0.05 mm. for the b-axis.

The structure factors were put on an approximately absolute scale by Wilson's method.²⁾ The temperature factor for all those atoms were taken as 2.2 Å^2, except for the bromine atom. McWeeny's f-curves³⁾ were employed for the light atoms, while that of Thomas⁴⁾ was used for the bromine atom.

Determination of the Structure

Since the c-axis is the shortest, it was expected that the projection of the structure on the (0 0 1) plane would show the maximum resolution of the molecular structure. This projection belongs to the same plane group pgg, no matter whether the space group is $Pna2_1$ or Pnam. Patterson syntheses were carried out using intensities of both the ordinary and modified types, the latter having been described by Takaki et al.⁵⁾

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1) M. Terao, K. Karasawa, N. Tanaka, H. Yonehara and H. Umezawa, *J. Antibiotics, Ser. A*, **13**, 401 (1960).

2) A. J. C. Wilson, *Acta Cryst.*, **2**, 318 (1949).

3) R. McWeeny, ibid., **4**, 513 (1951).

4) L. H. Thomas and K. Umeda, *J. Chem. Soc.*, **1957**, 293.

5) Y. Takaki, T. Oda and M. Sakata, Japan Applied Physics 9th Conference, 1962.

Fig. 1. Patterson projection on the (0 0 1) plane, ordiary type, P(UV).

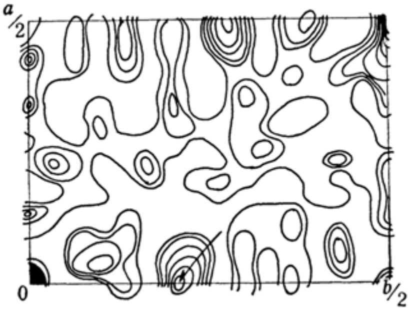

Fig. 2. Patterson projection on the (0 0 1) plane, modified type, P'(UV), as $b=3$.

$$P'(\mathrm{UV})=\sum\sum F^2(1-e^{-b(\sin\theta/\lambda)^2})^2$$
$$\times\cos 2\pi hx\cdot\cos 2\pi ky$$

These projections showed that the bromine-bromine vector lay very close to the b-axis. The approximate coordinates of the bromine atom was found to be:

$$x=0.0$$
$$y=0.1$$

Modified Fourier projections of the structure on the (0 0 1) plane were calculated, using the signs derived from the position of the heavy atom alone.

$$\rho'(xy)=\sum\sum F(1-e^{-b(\sin\theta/\lambda)^2})$$
$$\times\cos 2\pi hx\cdot\sin 2\pi ky$$

It was prepared with 56 terms (47% of the total reflection), using cosine terms only, as the assumed position of the bromine atom was on the b-axis.

Several models of the molecular structure fit the Fourier projection. However, it contains false peaks because all the sine terms were disregarded. Closer examination showed that the bromine atom was situated slightly out of the b-axis. By the trial and error method, the coordinates of the bromine atom were assigned to the following positions:

$$x=0.010$$
$$y=0.105$$

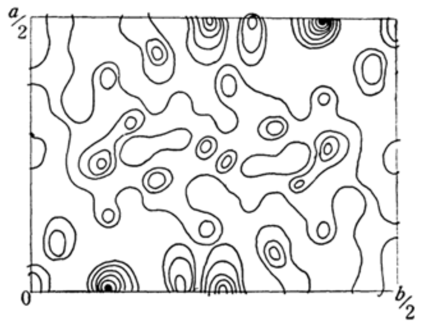

Fig. 3. Modified Fourier projection on the (0 0 1) plane.

A modified Fourier map was synthesized based on the new coordinates of the bromine atom, terms of doubtful sign being omitted. The number of terms used in this calculation was 107 out of a total of 121 observed reflections.

This Fourier projection indicates that the molecule has a six-membered ring system, with the replacements at the 1, 2 and 3 position and with position 2 taking the bromine atom, although it was not possible to distinguish carbon, nitrogen and oxygen atoms. The contribution of the light atoms were then included to determine the phases of the sturcture factors. In this calculation, all the atoms except bromine were treated as nitrogen atoms. The temperature factors for all those light atoms were taken as 2.2Å^2, and that of bromine, as 1.5Å^2.

In the second Fourier map, the locations of the oxygen atoms were easily recognized from the peak heights in the electron density distribution. This was also supported by the *R*-factor, when oxygen atoms were placed at positions 1 and 3. The six compounds listed below were considered (Fig. 4.), which are the six-membered ring systems of the meta substituents for the oxygen atoms. Compound III is already known,[6] and compound IV is difficult to fit because of its chemical properties.

$D(x, y)$ differential syntheses were calculated for the refinements of the atomic coordinates and for the distinction between carbon and nitrogen atoms. The mean value of the scattering factors of carbon and nitrogen was used for all the atoms in the pyrazine ring. These projections clearly showned the presence of anisotropy in the thermal vibrations of the bromine atom in the direction of the maximum amplitude lying almost parallel to the a-axis. The reliability factor became 18% when a correction of anisotropic temperature factors

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[6] G. W. Kenner, B. Lythgoe, A. R. Todd and A. Topham, *J. Chem. Soc.*, **1943**, 388.

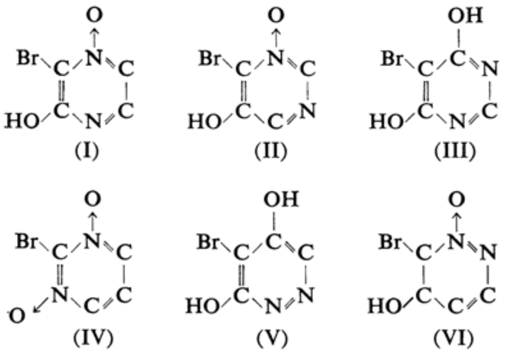


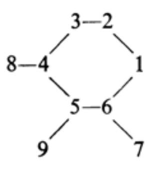
Fig. 4. Six possible structure formulae, as expected.

to bromine was made. To distinguish nitrogen from carbon atoms, $D(x,y)$ syntheses were carried out four times (Fig. 5). One can see that positions 1 and 4 have higher electron densities than those at positions 2, 3, 5 and 6. This suggests that positions 1 and 4 are occupied by nitrogen atoms. Furthermore, we have made structure factor calculations based on the following models:

Fig. 5. Differential synthesis, on the (0 0 1) plane.

Table I. R-Factor calculations for four estimated models

Model No.	Br	O	N	C	R-Factor %
1	9	7,8	1,2	3,4,5,6	17.5
2	9	7,8	1,3	2,4,5,6	17.7
3	9	7,8	1,4	2,3,5,6	15.9
4	9	7,8	2,4	1,3,5,6	16.9



From these calculations also, the best model is found to be the one in which nitrogen atoms occupy positions 1 and 4.

The peak heights of electron density in the final Fourier synthesis is shown to be seven electrons per Å^2 for positions 1 and 4, and six electrons per Å^2 for positions 2, 3, 5 and 6. Thus, from these three results, we concluded that nitrogen atoms occupy positions 1 and 4. The final reliability factor reached 12.3% for $(h\,k\,0)$.

The systematic absences required as a possible space group Pna2_1 or Pnam. However, the fact that four molecules in the unit cell combined with the following face, suggested that the space group in Pna2_1. If it is Pnam, molecules are required to lie on the mirror planes, which are 2.67 Å appart. Judging from the (0 0 1) projection, it seems very unlikely for the molecules to have such an arrangement. Furthermore, Wilson's test revealed that the space group has no center of symmetry. The space group was confirmed later by structrure factor calculations.

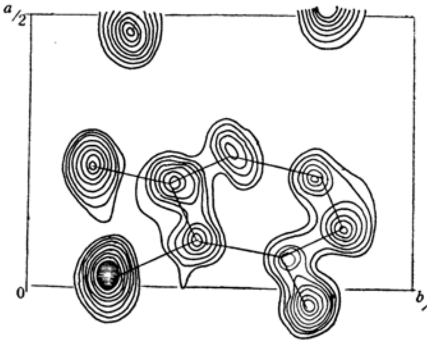


Fig. 6. Final Fourier map of bromoemimycin contours are drawn at intervals of 1 eÅ^2 except for bromine.

Now that the y-coordinate of the bromine atom was obtained by the (0 0 1) projection, and since the space group of the crystal Pna2_1 allowed the z-coordinate of this atom to be given at 0, we carried out Fourier analysis $\rho(x,y)$ based on the phases given by bromine. Although this projection contained overlappings of the molecule, it was possible to assign the z-coordinates of the light atoms by referring to the y-coordinates already obtained by the (0 0 1) projection. Two cycles of $D(y,z)$ syntheses were carried out for the refinements. The reliability factor for $(0\,k\,l)$ became 14.3%.

The observed and calculated structure factors are shown in Table II for $(h\,k\,0)$, and in Table III for $(0\,k\,l)$.

The final coordinates of the carbon, nitrogen, oxygen and bromine atoms are shown in Table IV.

TABLE II. COMPARISON OF CALCULATED AND OBSERVED STRUCTURE FACTORS (0 0 1).
Values are multiplied by 1/4

h k l	F_o	F_c	*h k l*	F_o	F_c	*h k l*	F_o	F_c	*h k l*	F_o	F_c
0 0 0	—	89	2 11 2	3.0	2.4	5 1 0	13.0	15.5	7 9 0	5.3	5.4
2	4.8	−3.3	12	3.1	−4.0	2	8.1	10.6	10	2.1	1.1
4	32.9	−35.9	13	2.9	2.9	3	4.8	−5.5	11	1.8	1.6
6	21.6	−20.7	14	7.8	−8.3	4	6.3	7.0			
8	11.6	11.3	15	0.0	−0.8	5	14.0	−14.0	8 0 0	5.6	4.2
10	14.7	15.8				6	6.4	−7.2	1	0.0	−1.0
12	0.0	−0.2	3 1 0	14.7	16.8	7	3.4	−3.4	2	2.7	2.6
14	10.0	−9.6	2	1.8	2.7	8	5.0	−5.2	3	2.2	2.3
			3	3.2	−3.4	9	6.5	6.3	4	3.7	−2.9
1 1 0	16.3	16.0	4	4.3	−3.1	10	1.4	2.0	5	0.0	−1.0
2	4.4	3.4	5	22.2	−23.5	11	8.3	−8.1	6	6.3	−6.9
3	5.5	−5.4	6	4.0	−5.0	12	3.5	2.9	7	1.7	−2.4
4	1.0	−0.7	7	2.8	−1.8	13	3.5	−4.8	8	3.4	3.4
5	15.6	−17.8	8	2.9	−2.1				9	0.0	−0.6
6	3.5	2.3	9	13.5	−15.8	6 0 0	6.7	6.2	10	3.6	3.5
7	7.4	−6.5	10	8.5	8.0	1	5.7	5.7			
8	2.4	−1.6	11	5.1	5.5	2	4.4	4.3	9 1 0	3.0	4.0
9	16.7	15.8	12	0.0	0.0	3	5.1	5.4	2	3.5	4.4
10	3.7	−2.6	13	7.8	−8.0	4	10.0	−10.5	3	2.7	−2.6
11	7.8	8.0	14	2.3	−1.3	5	2.6	−0.8	4	3.5	3.0
12	2.1	1.1				6	3.4	−2.7	5	2.7	−3.1
13	8.1	−7.8	4 0 0	26.2	24.2	7	2.3	−3.5	6	5.1	−5.2
14	0.0	0.2	1	1.0	−1.0	8	4.1	4.5	7	0.0	0.1
15	5.2	−7.2	2	3.6	3.7	9	2.4	−3.1	8	1.3	−1.5
			3	1.3	0.1	10	6.2	6.7	9	0.0	1.9
2 0 0	21.8	21.8	4	16.0	−16.4	11	2.5	3.0			
1	17.5	17.0	5	2.3	1.9	12	1.8	−1.7	10 0 0	2.3	3.2
2	7.7	5.7	6	9.5	−10.7				1	1.0	1.5
3	7.1	6.4	7	4.4	−3.8	7 1 0	6.5	5.9	2	0.7	1.4
4	24.0	−24.3	8	9.5	9.4	2	6.6	8.2	3	3.0	4.5
5	0.6	2.1	9	0.0	−0.7	3	3.8	−4.0	4	2.0	−1.9
6	6.7	−6.0	10	7.4	8.3	4	2.0	2.0	5	0.0	0.8
7	3.2	−3.3	11	1.2	2.9	5	7.1	−7.1	6	1.4	−1.4
8	11.7	11.1	12	0.0	−1.0	6	3.7	−4.6			
9	5.7	−5.3	13	2.9	2.6	7	2.3	2.7	11 1 0	0.0	1.3
10	10.9	11.5	14	5.3	−7.2	8	3.4	−3.3	2	0.0	0.5

TABLE III. COMPARISON OF CALCULATED AND OBSERVED STRUCTORS (1 0 0)
Values are multiplied by 1/4

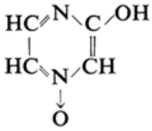
h k l	F_o	F_c	*h k l*	F_o	F_c	*h k l*	F_o	F_c	*h k l*	F_o	F_c
0 1 1	16.6	22.2	0 6 2	13.9	15.7	0 11 3	11.2	9.1	0 1 5	11.8	8.1
3	17.6	22.3	8	16.3	15.1	13	8.1	7.3	3	9.7	9.8
5	12.5	11.7	10	15.5	11.5				5	2.6	2.2
7	18.9	16.5	12	6.1	4.5	0 0 4	14.0	13.0	7	4.5	6.4
9	8.5	4.0	14	4.4	4.0	2	4.1	2.2	9	3.4	3.0
11	15.8	12.4				4	14.4	11.8			
13	5.4	5.8	0 1 3	11.2	11.4	6	9.2	8.9	0 0 6	8.8	10.7
			3	22.2	24.0	8	9.2	11.2	2	2.2	1.8
0 0 2	28.5	30.6	5	6.3	9.4	10	9.8	7.9	4	5.7	7.4
2	9.6	8.2	7	16.3	14.9	12	4.6	5.3	6	2.8	5.3
4	22.5	23.4	9	8.8	6.7						

TABLE IV. FRACTIONAL ATOMIC COORDINATES

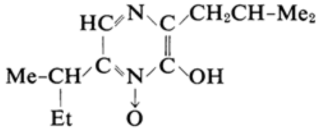
Atom	x	y	z
Br	0.014	0.106	0.000
O_1	−0.043	0.363	0.055
O_2	0.223	0.081	0.395
N_1	0.114	0.409	0.330
N_2	0.190	0.182	0.362
C_1	0.062	0.329	0.186
C_2	0.090	0.218	0.195
C_3	0.245	0.265	0.500
C_4	0.203	0.381	0.516

Discussion of the Results

From the coordinates of the atoms obtained, the molecular structure of bromoemimycin was determined to be 2-bromo-3-hydroxypyrazine-*N*-oxide-1. Therefore, the emimycin should have the structure 3-hydroxyl-pyrazine-*N*-oxide-1.



It is interesting to see that the structure of emimycin resembled that of the antibiotic aspergillic acid.[7] The molecular formula of aspergillic acid is:



The pyrazine ring of the bromoemimycin is planar, and the equation of the best plane as determined by the methods of least squares is:

$$0.222x+y-0.820z-0.524=0$$

The displacements of the atoms within the ring and O_1, O_2 and Br from this plane are shown in Table V.

The molecule can be described as a planar pyrazine ring, but the oxygen of *N*-oxide is about 0.4 Å apart from the pyrazine ring plane. The other oxygen and the bromine atom departed slightly from its plane.

TABLE V. DISPLACEMENTS OF ALL ATOMS FROM THE PYRAZINE RING PLANE

Atom	Displacements, Å
C_1	0.04
C_2	−0.02
N_1	0.02
C_3	−0.03
C_4	0.06
N_2	−0.05
O_1	−0.17
O_2	−0.38
Br	0.11

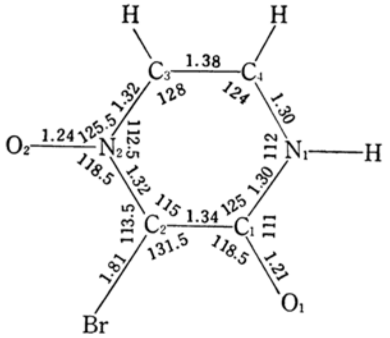


Fig. 7. The bond lengths and angles of the bromoemimycin.

The bond lengths and bond angles are given in Fig. 8.

The C_1–O_1 bond length 1.21 Å, appears to be significantly shorter than the reported value, 1.39 Å, for the $\rangle$C–OH bond in β-naphthol.[8] This might mean that the $\rangle$C=O bond here has a strong double bond character, so that the hydrogen atom may be attached to N_1. It may form a hydrogen bond with the carbonyl oxygen atom. It is expected that a keto-enol tautomerism similar to that found in the case of α-pyridone occurs.[9] The N_2–O_2 bond length is found to be 1.24 Å, which is the same as that reported[10] for the N–O bond in phenazine-5, 10-*N*-oxide. The C–Br bond length is 1.81 Å, which is considerably shorter than the reported value, 1.88 Å,[11] for *o*-bromo-benzoic acid. This suggests that the C–Br bond is associated with a double bond character. The C–N bonds within the pyrazine ring are considerably shorter than those reported for those in pyrazine, 1.334 Å,[12] and in pyrazinamide, 1.348 Å.[13] The C–C bonds are also shorter than the mean value for benzene, 1.39 Å.

The molecular arrangements are shown in Fig. 8 and Fig. 9.

The closest intermolecular interatomic distances occur between O_1 of molecule A and O_2' and N_2' of molecule B. The distances

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[7] G. T. Newbold, W. Sharp and F. S. Spring, ibid., **1951**, 2679.

[8] H. C. Watson and A. Hargreaves, *Acta Cryst.*, **11**, 556 (1958).

[9] B. R. Penfold, ibid., **6**, 591 (1953).

[10] Y. Namba, T. Oda and T. Watanabé, This Bulletin, **36**, 79 (1963).

[11] G. Fergson and G. A. Sim, *Acta Cryst.*, **15**, 346 (1962).

[12] P. J. Wheatley, ibid., **10**, 182 (1957).

[13] Y. Takaki, Y. Sasada and T. Watanabé, ibid., **13**, 693 (1960).

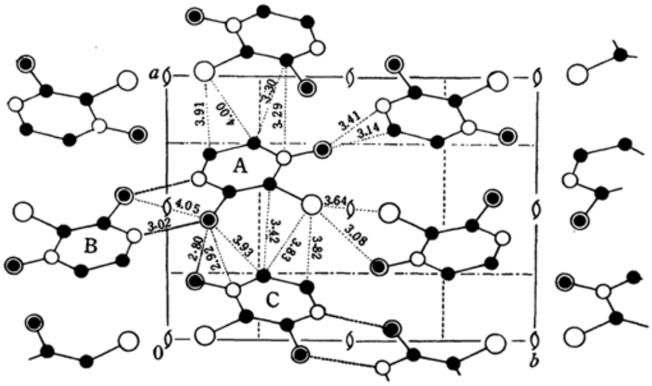

Fig. 8. Projection of the structure on (0 0 1). The hydrogen bonds are indicated by bold dotted lines. Other intermolecular atomic distances are shown by thin dotted lines.

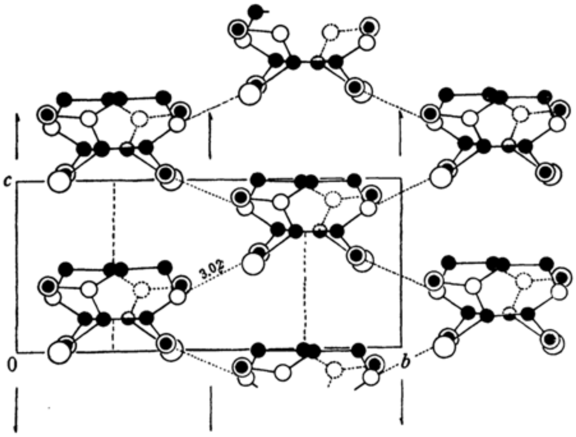

Fig. 9. The projection on (0 1 0), the hydrogen bonds are indicated by dotted lines.

are 2.81 Å for $O_1 \cdots O_2'$ and 2.92 Å for $O_1 \cdots N_2'$, and one might expect hydrogen bonds between those atom. However, there are no hydrogen atoms associated with these atoms, and the interatomic distances mentioned above may well be explained by the van der Waals contacts.

Another short interatomic distance, 3.02 Å, is found between N_1 of molecule A and O_1'' of molecule C. It appears that a hydrogen bond would exist between these atoms. If the tautomerism occurs, as has been suggested earlier, the molecular chain along the screw axis may be stabilized by the synchronized oscillation of the hydrogen atoms.[14]

The distance between O_2 and Br′, 3.08 Å, is considerably shorter than the accepted value of the van der Waals radii sum, 3.48 Å. It may not be possible to discuss this contact because of the inaccurate *z*-coordinates. Though we do not know the electronic state of the oxygen atom of the *N*-oxide group, nor the van der Waals radius of the atom in question, however, one might also suggest that this shortening is due to the charge-transfer effect.[15]

Another shorter intermolecular contact, 3.14 Å, occurs between the O_2 atom of the *N*-oxide group and the ring C_4 atom; this distance does not differ significantly from the reported value in *N*-oxyphenazine, 3.11 Å.[16]

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[14] M. L. Huggins, *Nature*, **139**, 550 (1937).

[15] T. L. Charlton and J. Trotter, *Acta Cryst.*, **16**, 313 (1963).

[16] R. Curti, V. Riganti and S. Loechi, ibid., **14**, 133 (1961).

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