# Crystal and Molecular Structure of Mitomycin C, an Anticancer Antibiotic

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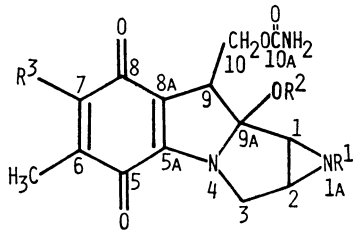
The crystal structure of mitomycin C was determined by means of X-ray diffraction. Mitomycin C was crystallized in the orthorhombic space group $P2_12_12_1$, with $a=12.887$, $b=13.851$, $c=9.718$ Å, and $Z=4$, and its structure solved by the trial and error method using the rigid-body approximation. The absolute configuration was determined by referring to that of *N*-(*p*-bromobenzoyl)mitomycin C. The carbamoyloxymethyl side chain participates in hydrogen bonds as in the case of other mitomycins in the crystalline state.

Mitomycins extracted from Streptomyces species, known to be antibiotics having anticancer activity, have the chemical structures shown in Fig. 1. Mitomycins were found to crosslink to double-stranded DNA by covalent bonds and to inhibit a duplication of DNA.[1] Szybalski and Iyer proposed that atoms C1 and C10 of mitomycin bind covalently to atoms O6 of guanine bases of the DNA duplex after enzymic reduction.[2] An intercalative model of mitomycin with DNA in the form of a semiquinone radical followed by the covalent bond formation was proposed by Tomatz *et al.*,[3] who stated that mitomycin might interact specifically with double-stranded polynucleotides, since no complex formation of the antibiotic with monomeric nucleotides or guanylyl(3′,5′)cytidine was observed. A recent CD study showed that mitomycin might interact with the fragment having the GpC(deoxyguanosine-phosphate-deoxycytidine) base sequence in DNA.[4]



| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| mitomycin A | H | $CH_3$ | $OCH_3$ |
| *N*-methyl-mitomycin A | $CH_3$ | $CH_3$ | $OCH_3$ |
| mitomycin B | $CH_3$ | H | $OCH_3$ |
| mitomycin C | H | $CH_3$ | $NH_2$ |
| porfiromycin | $CH_3$ | $CH_3$ | $NH_2$ |
| 7-hydroxyl-porfiromycin | $CH_3$ | $CH_3$ | OH |

Fig. 1. Structures of mitomycins and porfiromycins.

This paper deals with the three-dimensional structure analysis of mitomycin C, and a structural comparison with *N*-brosylmitomycin A,[5] 7-demethoxy-7-(*p*-bromoanilino)mitomycin B[6] and *N*-(*p*-bromobenzoyl)mitomycin C.[7]

## Experimental

Mitomycin C crystallized in the form of dark violet plates from a 50% aqueous ethanol solution. Oscillation and Weissenberg photographs showed the crystal to be orthorhombic with the space group $P2_12_12_1$. The crystallographic data are given in Table 1. The density measurement made by a floatation method using a benzene–carbon tetrachloride mixture revealed that an asymmetric unit contains one mitomycin C and two water molecules.

Table 1. Crystal data

| | |
|---|---|
| Formula | $C_{15}H_{18}N_4O_5\cdot 2H_2O$ |
| Formula weight | 370.37 |
| Crystal system | Orthorhombic |
| Space group | $P2_12_12_1$ |
| $a$ | 12.887(2) Å |
| $b$ | 13.851(2) Å |
| $c$ | 9.718(1) Å |
| $V$ | 1734.6(3) Å$^3$ |
| $Z$ | 4 |
| $D_m$ | 1.423(4) Mg/m$^3$ |
| $D_x$ | 1.418 Mg/m$^3$ |

Three-dimensional intensity data were collected on a computer-controlled four-circle diffractometer using Ni-filtered Cu $K\alpha$ radiation. Employing $\omega$-$2\theta$ scan technique with a scan speed of 4°/min($2\theta$) and 10 s. background measurement at each end of the scan, 1145 independent structure factor magnitudes greater than three times their standard deviations were obtained within $\sin\theta/\lambda=0.547$ Å$^{-1}$.

## Structure Determination and Refinement

Orientation of the molecule in the crystal was determined by the rigid group convolution and search method (RICS).† The coordinates of 17 atoms involving ring portion in 7-demethoxy-7-(*p*-bromoanilino)mitomycin B solved by Yahashi and Matsubara[6] were utilized as a model. The convolution calculated from the model having the benzoquinone ring vertical to the b-axis showed good agreement with the three-dimensional Patterson peaks around the origin. However, the translation search procedure failed to find the correct position in the crystal by employing the resultant model. This might be due to the peculiar molecular packing. Thus the R-map method was tried using a program written by one of the authors (K. O.). The minimum *R*-value (0.51) was obtained by translating the model having the C5A–C6 vector parallel to the c-axis. Using the coordinates of 17 atoms obtained by the above method, an electron density map was constructed to find all nonhydrogen atoms.

† The rigid group convolution and search programs were written by Dr. I. Tanaka.

Table 2. Final positional and thermal parameters with their estimated standard deviations in parentheses ($\times 10^4$)

Anisotropic temperature factors are of the form;
$\exp[-(B_{11}h^2+B_{22}k^2+B_{33}l^2+B_{12}hk+B_{13}hl+B_{23}kl)]$.

| | $x$ | $y$ | $z$ | $B_{11}$ | $B_{22}$ | $B_{33}$ | $B_{12}$ | $B_{13}$ | $B_{23}$ |
|---|---|---|---|---|---|---|---|---|---|
| C1 | 3874(7) | 5655(7) | 2883(10) | 43(6) | 33(5) | 91(12) | 16(11) | 0(16) | 8(15) |
| C2 | 5019(8) | 5500(7) | 3045(10) | 48(6) | 42(6) | 77(11) | 1(11) | 28(16) | 26(15) |
| C3 | 5463(7) | 6292(7) | 3958(11) | 32(6) | 49(6) | 87(12) | 4(11) | 31(16) | −14(15) |
| C5A | 4194(6) | 6769(6) | 5880(9) | 29(5) | 30(5) | 40(9) | −4(9) | 8(12) | 0(12) |
| C5 | 4919(7) | 6867(7) | 7043(9) | 32(5) | 46(6) | 70(11) | 1(10) | −7(15) | 16(15) |
| C6 | 4464(7) | 6941(7) | 8441(9) | 39(5) | 44(6) | 62(10) | −12(11) | −18(15) | 17(14) |
| C7 | 3392(7) | 6941(6) | 8564(9) | 40(6) | 35(5) | 54(10) | 3(10) | −11(14) | −8(13) |
| C8 | 2681(7) | 6841(6) | 7341(9) | 34(5) | 33(5) | 58(10) | −9(9) | −10(14) | 11(13) |
| C8A | 3141(6) | 6741(6) | 6013(9) | 32(5) | 34(5) | 40(9) | −3(9) | −6(13) | 1(12) |
| C9 | 2631(8) | 6610(7) | 4615(9) | 30(6) | 39(6) | 51(9) | −1(10) | −8(13) | 3(13) |
| C9A | 3602(7) | 6587(6) | 3654(8) | 44(6) | 26(5) | 35(9) | 0(9) | −9(13) | −13(11) |
| C10 | 1965(7) | 5688(8) | 4636(10) | 42(7) | 54(6) | 57(10) | −29(12) | −27(14) | 3(16) |
| C10A | 497(7) | 5631(7) | 3125(10) | 34(6) | 50(6) | 75(11) | −3(11) | −9(15) | −19(16) |
| C11 | 5155(8) | 7029(9) | 9667(10) | 53(7) | 80(8) | 58(11) | −23(14) | −41(16) | 22(18) |
| C12 | 3544(9) | 8296(7) | 3020(11) | 84(9) | 35(5) | 101(13) | −20(13) | −13(21) | 3(16) |
| N1A | 4255(6) | 4882(6) | 3835(9) | 50(6) | 45(5) | 98(11) | 4(9) | −16(14) | −1(13) |
| N4 | 4503(5) | 6733(5) | 4550(8) | 27(4) | 39(4) | 63(9) | 0(8) | −3(11) | 1(11) |
| N7 | 2876(7) | 7051(7) | 9746(8) | 53(6) | 68(6) | 56(9) | −1(11) | 12(13) | −1(13) |
| N14 | 177(6) | 5515(6) | 1800(8) | 51(6) | 55(5) | 72(9) | 1(10) | −21(13) | 1(11) |
| O5 | 5859(5) | 6915(5) | 6873(7) | 36(4) | 74(5) | 91(9) | −21(8) | −16(11) | 3(12) |
| O8 | 1734(4) | 6830(5) | 7548(7) | 35(4) | 51(4) | 70(7) | 8(7) | 11(10) | 0(10) |
| O9 | 3553(5) | 7289(4) | 2583(6) | 56(4) | 31(3) | 61(7) | 2(7) | −11(11) | 10(9) |
| O10 | 1531(5) | 5584(5) | 3218(6) | 35(4) | 59(4) | 59(7) | −11(8) | −8(10) | −14(10) |
| O13 | −68(6) | 5776(6) | 4093(8) | 58(5) | 99(7) | 90(9) | 7(11) | −6(12) | −59(14) |
| OW1 | −2172(6) | 5436(7) | 3855(9) | 54(5) | 104(7) | 147(11) | −19(11) | 30(15) | −13(16) |
| OW2 | −2111(7) | 5671(7) | 935(9) | 87(7) | 71(6) | 161(12) | 1(12) | −48(17) | 10(16) |

The structure was refined by the full-matrix least-squares method with isotropic temperature factors, and then by the block-diagonal least-squares method with anisotropic temperature factors for all nonhydrogen atoms. At this stage, difference Fourier synthesis was carried out, the result being unsatisfactory for full detection of hydrogen atoms. At the final refinement, following weighting scheme was employed; $w=0.0$ for $F_o=0.0$, $w=1.0$ for $F_o \leqq 45.0$, and $w=1.0/(1.0+0.235(F_o-45.0))$ for $F_o \leqq 45.0$. The final $R$-value is 0.080 for all reflections. The final atomic parameters are given in Table 2 with their estimated standard devia-

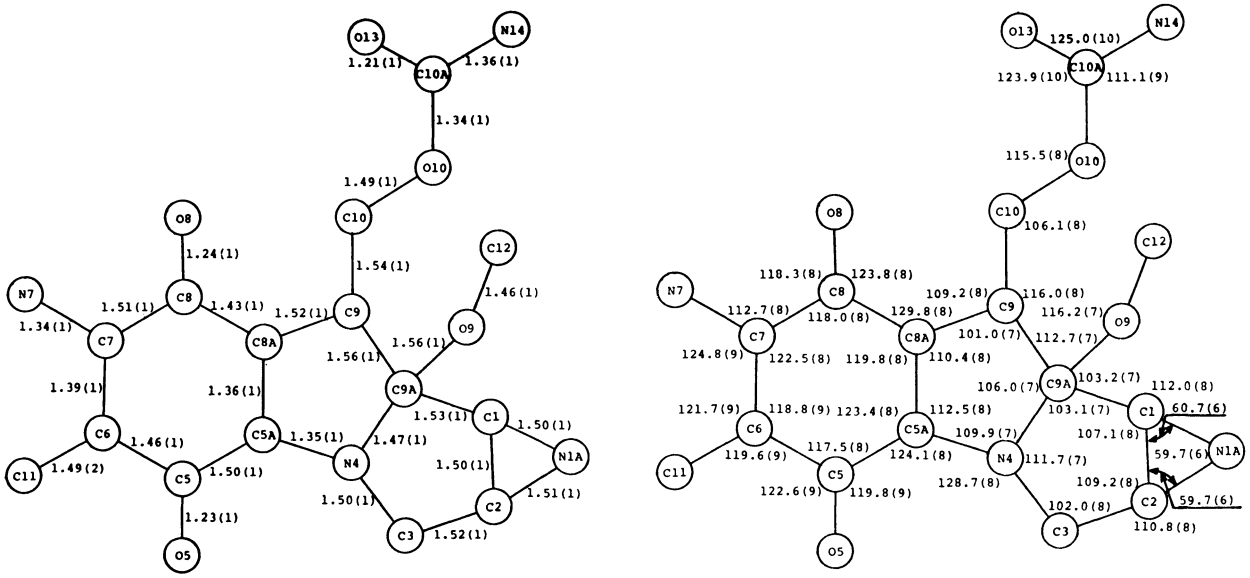



Fig. 2. Bond lengths/Å and angles/° with their estimated standard deviations in parentheses.

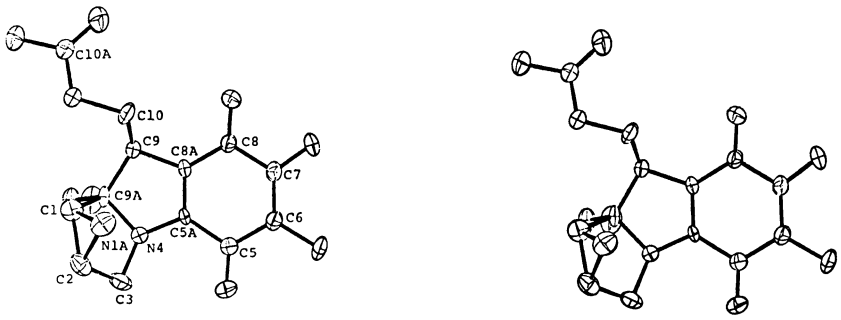



Fig. 3. Stereoscopic view of mitomycin C molecule.

tions. The observed and calculated structure factors are given in Table 3.†† The absolute configuration of mitomycin C was determined by referring to that of *N*-(*p*-bromobenzoyl)mitomycin C.[7]

The computations were performed on a NEAC 2200-700 computer at the Computing Center of this University, using Universal Crystallographic Computing System-Osaka.[8]

## Results and Discussion

The bond lengths and angles are given in Fig. 2. A stereoscopic view of the mitomycin C molecule is shown in Fig. 3. The molecular geometry is essentially similar to that of the bromo derivative of mitomycin A,[5] exclusive of the conformation of the carbamoyloxymethyl side chain. The torsion angles of the side chain in four kinds of mitomycins are shown in Table 4. The variance among these torsion angles may be due to the difference in packing force acting on each molecule in the crystal, suggesting that the group is flexible in solution. The fact that the carbamoyl group of the side chain participates in intermolecular hydrogen bond formations, and the configuration of C9(R) is invariant in all cases,[5-7] seems to give valuable information on the interaction mechanism of mitomycin with DNA.

The strain of the indolequinone ring in mitomycins is dependent on the side chain configurations at positions C9 and C9A, *viz.*, the trans-arrangement of carbamoyloxymethyl and methoxy groups in mitomycins A and C

Table 4. Torsion angles in carbamoyloxymethyl side chain

| | $\theta$ (C8A–C9–C10–O10) | $\phi$ (C9–C10–O10–C10A) | $\psi$ (C10–O10–C10A–O13) |
|---|---|---|---|
| Br-A | 179.5° | 166.2° | 1.8° |
| Br-B | 298.5 | 160.1 | 344.8 |
| Br-C | 180.5 | 280.7 | 353.9 |
| C | 182.4 | 244.0 | 2.3 |

a) Angles are measured in the clockwise sence, faking the cis position as 0°. The notations $\theta$, $\phi$, and $\psi$ are shown in Fig. 5. Br-A; *N*-brosylmitomycin A[5] Br-B; 7-demethoxy-7-(*p*-bromoanilino)mitomycin B[6] Br-C; *N*-(*p*-bromobenzoyl)mitomycin C[7] C; mitomycin C.

†† Table 3 is kept as a Document at the Office of The Chemical Society of Japan(Document No. 7925).

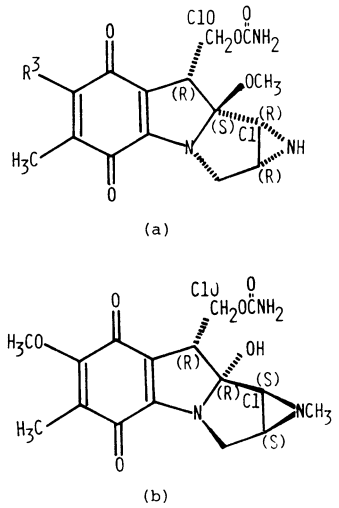



Fig. 4. Absolute configurations.
(a) Mitomycins A and C, (b) mitomycin B.

may release the intramolecular short contacts between these groups. The least-squares plane through the indolequinone ring including atoms C11 and N7 is represented by $-0.035x-0.994y-0.106z+5.225=0.0$. The mean deviation of the atoms from this plane is 0.024 Å, smaller than in mitomycin B (0.079 Å)[6] and close to that in mitomycin A(0.026 Å).[5] The configurations at C9A, C1, and C2 are, respectively, (S), (R), and (R) in mitomycins A[5] and C,[7] differing from those in mitomycin B[6] as shown in Fig. 4. Consequently, significant differences can be seen between the molecular structures of mitomycin B and mitomycins A or C,

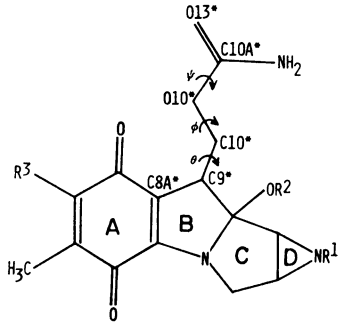



Fig. 5. Notations of torsion angles in carbamoyloxymethyl side chain and of the least-squares planes.

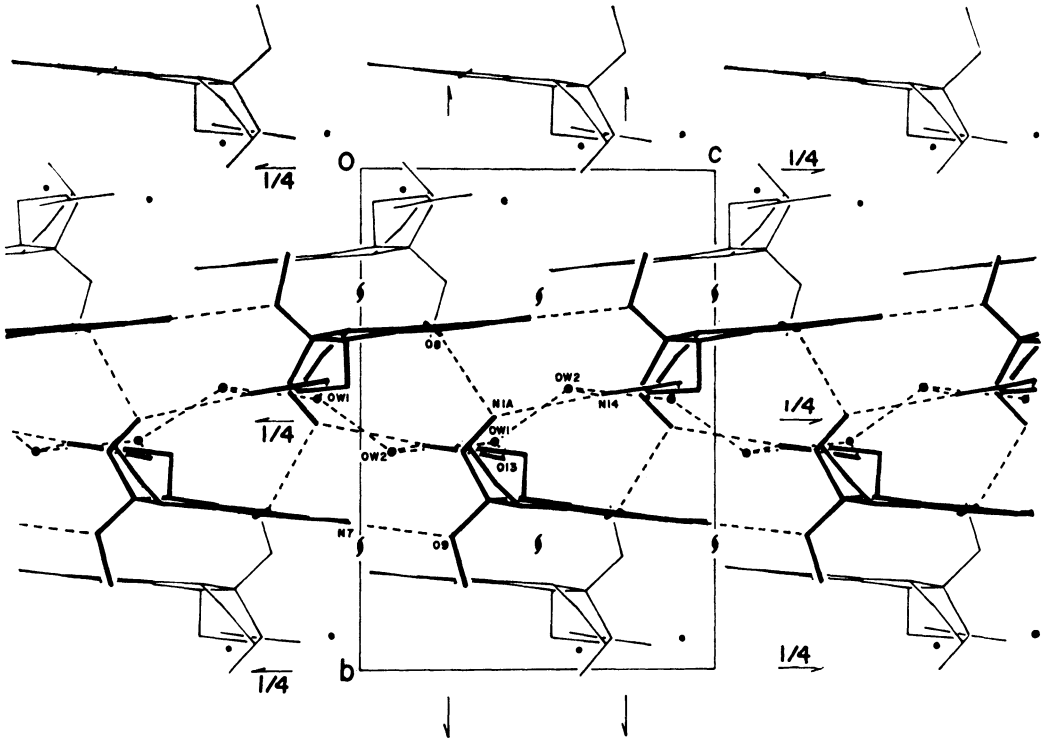



Fig. 6. Crystal structure of mitomycin C projected along the a-axis. Dashed lines and black circles indicate the hydrogen bonds and water oxygen atoms, respectively.

Table 5. Dihedral angles between the least-squares planes

| Plane | Plane | Br-A[5] | Br-B[6] | Br-C[7] | C |
|---|---|---|---|---|---|
| A | B | 3.7° | 9.9° | 4.4° | 3.0° |
| A | C | 133.9 | 115.7 | 137.8 | 135.3 |
| A | D | 54.0 | 41.0 | 57.4 | 54.8 |
| B | C | 131.1 | 123.5 | 133.4 | 134.2 |
| B | D | 53.4 | 47.2 | 54.4 | 53.5 |
| C | D | 98.4 | 100.0 | 97.4 | 97.9 |

The notations A, B, C, and D are shown in Fig. 5.

Table 6. Hydrogen bond distances and short contacts

| Hydrogen bonds | | Short contacts | |
|---|---|---|---|
| O13–OW1 | 2.76 Å | C5A–N14[a] | 3.40 Å |
| N14–OW2 | 3.07 | C5–N14[a] | 3.30 |
| OW1–OW2 | 2.86 | O5–C10A[d] | 3.44 |
| O8–N1A[a] | 2.96 | O5–O9[d] | 3.40 |
| O9–N7[b] | 2.92 | C7–OW2[d] | 3.41 |
| N1A–N14[a] | 3.03 | N7–OW2[d] | 3.23 |
| OW1–OW2[c] | 2.70 | O13–C5[e] | 3.46 |

Symmetry codes;

a) $\frac{1}{2}-x, 1-y, \frac{1}{2}-z$ b) $x, y, -1+z$

c) $-\frac{1}{2}-x, 1-y, \frac{1}{2}+z$ d) $\frac{1}{2}+x, \frac{3}{2}-y, 1-z$

e) $-\frac{1}{2}+x, \frac{1}{2}-y, 1-z$

especially in the dihedral angles between the least-squares planes given in Table 5. For example, in mitomycins A and C, the dihedral angles between planes B and C and between B and D become larger than those in mitomycin B, probably to avoid the short contact between C1 and C10.

The crystal structure is shown in Fig. 6. The molecules at the positions $(x, y, z)$, $(x, y, 1+z)$, and $(1/2-x, -y, 1/2+z)$ are linked by seven intermolecular hydrogen bonds to form a double layered structure (Table 6 and Fig. 6). These layers are held together mainly by van der Waals forces. The indolequinone ring and carbamoyl group with atom O10 are almost vertical to the b-axis, the interplanar spacing being about one-eight of the b-axis dimesion.

A discussion on the interaction between mitomycin and the double-stranded DNA was given in a previous paper.[9] The intercalative model for the interaction of mitomycin with DNA could be built by using the space-filling model and the Kendrew-Watson skeletal model. As the atomic coordinates of base-pair and sugar-phosphate backbone of DNA, the structural data of B-DNA[10] and the crystalline complex of ethidium bromide (2,7-diamino-9-phenyl-10-ethylphenanthridinium bromide) and iodoCpG(5-iodocytidylyl(3′,5′)-guanosine)[11] were referred to.

Although a favorable intercalation model of mitomycin with the GpC fragment of the double-stranded DNA was obtained by examining the stacking and hydrogen bonding, further evidence is necessary before the validity of the intercalative model is discussed.

We thank Dr. Kazuo Yamaguchi for supplying atomic coordinates of *N*-(*p*-bromobenzoyl)mitomycin C.

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