X-Ray crystallographic analysis of 3-(2'-phenyl-2,4'-bithiazole-4-carboxamido)propyldimethylsulphonium iodide, an analogue of the DNA-binding portion of bleomycin A_2

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ABSTRACT

The crystal and molecular structure of the title compound, an analogue of the DNA binding region of bleomycin A_2, has been determined by X-ray crystallography. All the three independent molecules in an asymmetric unit are approximately planar with fully extended side chains.

A computer graphics model-building study has shown that the phenyl group and the second thiazole ring can be intercalated between the base pairs of the double-stranded deoxydinucleoside phosphate d(CpG), and also that the sulphonium cation can interact with a backbone phosphate group. This model is in accord with NMR spectral data.

INTRODUCTION

The bleomycins are a group of closely-related antibiotics useful in the treatment of various types of cancer, especially squamous cell carcinomas, testicular cancers and cancers of the head and neck (1). The drugs are believed to act by degrading cellular DNA and recent evidence suggests that the ultimate agent of DNA damage is some form of activated reduced oxygen, produced as a consequence of oxidation of bleomycin-chelated Fe(II) to Fe(III) in a quaternary DNA.bleomycin.iron.oxygen complex (1). The drug molecule itself may be divided into two regions; the DNA-binding portion, containing the bithiazole moiety and the terminal amine and the metal ion-chelating part, comprising most of the remainder of the molecule (figure 1).

Physical methods such as ESR (2-6), NMR (7-11) spectroscopy and single crystal diffraction (9) have been intensively used for the study of bleomycin itself and/or its components and biosynthetic precursors. However the structure of the activated bleomycin species is not known with certainty and the mechanism of the DNA damage is still at the stage of speculation. So far only one model metal complex has been reported which is biologically active, producing oxygen sensitive complex with Fe(II) at physiological values of pH (12).

NMR studies show (13,14) that the bithiazole and terminal amine are the moities most strongly associated with DNA. Whether the bithiazole intercal-

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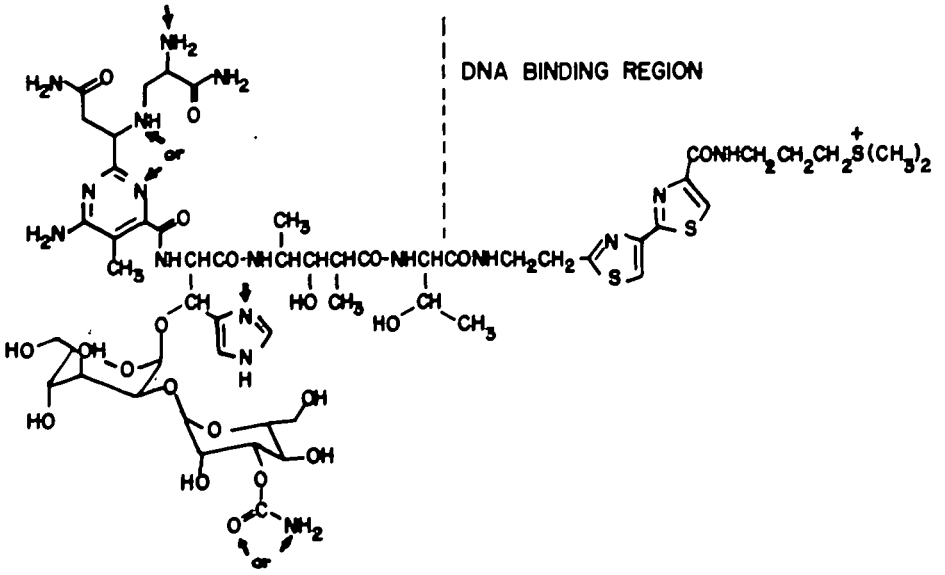

Figure 1. Structure of bleomycin A_2 showing the DNA binding region of the molecule. Arrows on the left indicate likely ligands for metal ion binding.

ates between DNA bases or simply binds in the minor groove is disputed. Bleomycin is reported to cause an increase in the length of DNA, and to unwind DNA in a manner characteristic of classical intercalators. In addition, electric dichroism measurements indicate that the bithiazole rings are parallel to the base pairs in DNA-bleomycin complexes (15,16). However NMR studies of the poly (dA-dT) .poly (dA-dT)-bleomycin A_2 complex show that the bithiazole protons do not experience the large upfield shifts normally associated with an intercalative interaction (14,17).

The terminal amine is believed necessary for the formation of an ionic bond between the drug and the negatively charged nucleic acid backbone so that the drug is brought in close proximity to its target. It is known that loss of the terminal amine (to give the acid) renders bleomycin inactive (18). Removal of the cationic charge of bleomycin A_2 to give the demethyl-A_2 derivative results in a decrease in the rate of DNA degradation, suggesting that the decrease in rate is the result of a lower frequency of interaction of the drug with the DNA (19,21).

Several compounds modelling the DNA binding region of bleomycin A_2 have been synthesized and their interactions with poly (dA-dT) . poly (dA-dT) have been investigated by NMR spectroscopy for the purpose of designing synthetic

derivatives of bleomycin. Derivatives of 2-phenylthiazole-4-carboxylic acid and 2'-phenyl-2,4'-bithiazole-4-carboxylic acid containing the 3-aminopropyl-dimethyl-sulphonium side chain of bleomycin A_2 were found to intercalate in poly (dA-dT) .poly (dA-dT) to a much greater extent than 2'-alkyl substituted 2,4'-bithiazole derivatives structurally similar to the parent compound. These data show that steric constraints play an important role in the binding of the parent bleomycins to DNA; furthermore, the data suggest the possibility that the interaction of bleomycin analogues could be manipulated by appropriate choice of side chain substituents and/or intercalating groups. In the present study, the structure of the phenylbithiazole analogue has been studied by X-ray crystallography, and its possible interaction with a DNA fragment examined by model-building. These studies provide information about the possible nature of complexes of these analogues with DNA. Ultimately, it is hoped that they will enable the rational design of DNA ligands having specific binding properties.

EXPERIMENTAL

The preparation of the compound is described elsewhere. The material was obtained as fine needles upon crystallization from a mixture of methanol and water.

Accurate cell dimensions were obtained by measurement of 25 θ values on an Enraf-Nonius CAD4 diffractometer, after a preliminary examination by Weisenberg photography. The intensity data were collected on the diffractometer with Cu-Kα radiation, operated in the θ-2θ scan mode up to θ=50°. A periodic check on intensities data were corrected anisotropically for decomposition. The maximum and average correction factors were 1.25 and 1.08, respectively.

Crystal Data:- $C_{18}H_{20}IS_3N_3O$. Molecular Weight 517.48, triclinic, a = 5.434 (1), b = 36.129(2), c = 26.314(3)Å, α = 120.24(1), β = 97.86(1), γ = 86.06(1)°, U = 3179.5(1.6)Å^3, D = 1.612 g cm^{-3}, z = 6, F(000) = 1548, space group $P\bar{1}$, μ (Cu-Kα) = 147.7 cm^{-1}.

The crystal available for the data collection was very small (0.14 x 0.02 x 0.007 mm). Therefore the intensity data was measured with Cu-Kα radiation in spite of the high μ value. Out of 7515 observed reflections only 3373 satisfied $I \geq 1\sigma(I)$; 2573 reflections with $I \geq 3\sigma(I)$ were used for the structure refinement. As the intensity statistics showed a centric structure, the space group $P\bar{1}$ was adopted with three independent molecules per asymmetric unit. The structure was solved by the heavy-atom technique combined with

Table 1. Atomic coordinates with estimated standard deviations

ATOM	X	Y	Z
I1	0.3264(4)	0.30523(7)	0.50780(7)
I2	0.1230(4)	0.49228(8)	0.79745(7)
I3	0.8184(4)	0.20243(7)	0.69465(8)
S1	-0.198(2)	0.4725(3)	0.6497(3)
S2	0.358(2)	0.3209(3)	0.2588(3)
S3	0.944(2)	0.2148(3)	0.3516(3)
S4	0.150(2)	0.1770(3)	0.5277(3)
S5	1.098(2)	-0.0617(2)	0.6793(3)
S6	1.593(2)	0.1369(3)	0.7843(3)
S7	1.378(2)	0.6497(3)	0.1775(3)
S8	0.581(2)	0.2594(3)	-0.0614(3)
S9	0.192(2)	0.3518(3)	0.1365(3)
O1	-0.219(4)	0.4410(6)	0.3770(6)
O2	0.398(4)	-0.0637(6)	0.5592(6)
O3	1.161(4)	0.3768(7)	-0.0645(7)
N1	-0.059(4)	0.4049(7)	0.4361(7)
N2	0.294(4)	0.3410(7)	0.3620(7)
N3	0.768(4)	0.2441(7)	0.2759(7)
N4	0.505(4)	0.0312(7)	0.5952(7)
N5	0.924(4)	0.0209(7)	0.6592(7)
N6	1.490(4)	0.0311(7)	0.7555(7)
N7	1.090(4)	0.4360(7)	0.0320(7)
N8	0.729(4)	0.3616(7)	0.0208(7)
N9	0.252(4)	0.2738(7)	0.0313(7)
C1	-0.475(7)	0.4940(12)	0.6915(12)
C2	-0.143(5)	0.3998(10)	0.6354(10)
C3	-0.331(5)	0.4616(9)	0.5782(9)
C4	-0.138(5)	0.4348(9)	0.5336(9)
C5	-0.235(5)	0.4354(9)	0.4778(9)
C6	-0.072(5)	0.4099(8)	0.3890(9)
C7	0.114(5)	0.3745(9)	0.3490(9)
C8	0.113(5)	0.3676(9)	0.2939(9)
C9	0.431(5)	0.3116(9)	0.3209(9)
C10	0.624(5)	0.2748(10)	0.3224(9)
C11	0.705(5)	0.2632(9)	0.3688(9)
C12	0.939(5)	0.2106(8)	0.2831(8)
C13	1.103(5)	0.1727(9)	0.2380(9)
C14	1.060(5)	0.1659(9)	0.1832(9)
C15	1.220(6)	0.1300(10)	0.1424(10)
C16	1.428(6)	0.1020(10)	0.1564(10)
C17	1.460(5)	0.1116(9)	0.2123(9)
C18	1.319(6)	0.1474(10)	0.2559(10)
C19	-0.154(6)	0.1949(11)	0.5070(11)
C20	0.225(6)	0.2348(11)	0.5997(11)
C21	0.089(6)	0.1174(10)	0.5391(10)
C22	0.327(5)	0.0976(9)	0.5639(9)
C23	0.290(6)	0.0436(10)	0.5649(10)
C24	0.538(5)	-0.0209(9)	0.5909(9)
C25	0.769(5)	-0.0253(9)	0.6264(9)
C26	0.825(5)	-0.0735(9)	0.6321(9)
C27	1.112(5)	0.0090(9)	0.6882(9)
C28	1.311(5)	0.0480(9)	0.7254(9)
C29	1.334(5)	0.1036(9)	0.7362(9)
C30	1.644(5)	0.0734(9)	0.7888(9)

Table 1. (continued)

C31	1.869(5)	0.0684(9)	0.8275(9)
C32	2.059(5)	0.1096(10)	0.8551(10)
C33	2.252(5)	0.1005(9)	0.8883(9)
C34	2.268(6)	0.0539(10)	0.8976(10)
C35	2.073(6)	0.0140(11)	0.8721(11)
C36	1.880(5)	0.0199(9)	0.8330(9)
C37	1.654(6)	0.6900(11)	0.1970(11)
C38	1.370(6)	0.6354(11)	0.2340(11)
C39	1.451(5)	0.5781(9)	0.1170(9)
C40	1.238(5)	0.5363(9)	0.1010(9)
C41	1.277(5)	0.4772(9)	0.0437(9)
C42	1.057(5)	0.3881(9)	-0.0220(9)
C43	0.863(5)	0.3464(9)	-0.0260(9)
C44	0.803(6)	0.2930(10)	-0.0744(10)
C45	0.582(5)	0.3208(9)	0.0096(9)
C46	0.411(6)	0.3200(10)	0.0465(10)
C47	0.405(6)	0.3703(10)	0.1063(10)
C48	0.141(5)	0.2839(9)	0.0735(8)
C49	-0.037(5)	0.2396(8)	0.0660(8)
C50	-0.201(5)	0.2557(8)	0.1091(8)
C51	-0.356(5)	0.2113(10)	0.0981(10)
C52	-0.365(6)	0.1538(10)	0.0527(11)
C53	-0.214(6)	0.1456(11)	0.0146(11)
C54	-0.051(6)	0.1856(10)	0.0191(10)

direct methods. Three iodine atoms were located from the Patterson map in conjunction with examination of the second most consistent E map. The subsequent difference Fourier map at R = 0.346 showed some strong peaks, out of which nine seemed to represent reasonable geometry for three sulphurs in each of three molecules. This assignment gave acceptable intermolecular contacts for the atoms, as well as being consistent with the vector map.

Least-squares refinement with the iodine and sulphur atoms converged to an R factor of 0.236 and the subsequent difference map revealed all but one carbon atom of molecule I but only half of the structure of molecules II and III. Several cycles of least-squares refinements including these located atoms reduced the R factor to 0.147 and the rest of the structure was revealed in the subsequent difference map. Least-squares refinement with anisotropic temperature factors for the iodine and sulphur atoms and isotropic temperature factors for the rest of the atoms converged to an R factor of 0.064. Amide hydrogen atoms and some of the CH_2 and CH_3 hydrogen atoms were located in a subsequent difference map. Accordingly these atoms were assigned their observed positions and the remaining ones were generated assuming a near-ideal geometry of the functional groups. The hydrogen atoms were included in the structure factor calculations but their parameters were not refined. The final R factor was 0.057. Unit weights were assigned to all

reflections. Table 1 gives the final atomic coordinates. Thermal parameters and structure factors are available on request from S.N.

Computer graphics analyses were made using programs written by S.A. Islam at King's College. These were performed on a Tektronix 4025A raster scan terminal linked to a PDP 11/34 computer. Coordinates for the deoxydinucleoside phosphate intercalative geometry were taken from the study of d(CpG) with proflavine (22). It has been shown elsewhere (23,24) that the backbone conformation of intercalated dinucleosides (whether ribo- or deoxy-) is approximately constant regardless of the nature of the interacting drug; this data set is the most accurate available for deoxydinucleoside-monophosphate - drug complexes.

RESULTS AND DISCUSSION

Molecular Structure: There are three independent molecules, I, II and III in an asymmetric unit. The geometry of the three molecules is very similar indicating that crystal packing forces do not dominate the conformation of this apparently rather flexible molecule. Average bond lengths and angles over the three molecules are given in Table 2 and averaged geometry is considered for much of the discussion which follows.

The molecule is approximately planar (figure 2). The two thiazole rings of bithiazole form an angle of 1.7° to each other for all three independent molecules and the electrons are therefore presumably delocalized over the two rings. This value compares closely with that of 6° in the bithiazole-containing component of bleomycin (25). The absence of 1' and 3' substituents on the bithiazole system allows the phenyl ring to be coplanar with the bithiazole rings. This is in contrast to biphenyl systems in which even hydrogen-hydrogen interactions suffice to skew the rings with respect to one another. The central C-C distance of a bithiazole is 1.41Å, averaged over the molecules and the two rings are *trans* to each other. The terminal phenyl ring is slightly rotated about its exocyclic bond, forming a dihedral angle of 10.2° to the adjacent bithiazole ring. The maximum deviation of a non-hydrogen atom from the bithiazole ring plane is 0.85Å for one of the terminal S-methyl carbon atom with the rest of the atoms only 0.03 - 0.36Å away from the plane. The peptide groups are planar but the hydrogen atoms are slightly off the plane: 0.44, 0.05, 0.28Å for molecules I, II and III respectively. This is due to an intramolecular interaction with the nitrogen atom of the first thiazole (N2...HN1 = 2.22Å, N5...HN4 = 2.15Å, N8...HN7 = 1.43Å). The torsion angles around the peptide bond averaged over the three molecules are included

Table 2. Average bond lengths (A) and angles (°), and peptide torsion angles for each molecule: numbering of atoms is for molecule I.

S1 - C1	1.80(1)	C1 - S1 - C2	103.3(6)
S1 - C2	1.73(1)	C1 - S1 - C3	102.4(6)
S1 - C3	1.80(1)	C2 - S1 - C3	102.6(5)
C3 - C4	1.53(1)	S1 - C3 - C4	110.3(8)
C4 - C5	1.50(1)	C3 - C4 - C5	111(1)
C5 - N1	1.43(1)	C5 - N1 - C6	119.6(9)
N1 - C6	1.32(1)	N1 - C6 - O1	126(1)
C6 - O1	1.22(1)	N1 - C6 - C7	113(1)
C6 - O7	1.50(1)	O1 - C6 - C7	121(1)
C7 - C8	1.36(1)	C6 - C7 - C8	123(1)
C8 - S2	1.74(1)	C6 - C7 - N2	121.9(9)
S2 - C9	1.75(1)	C7 - C8 - S2	110.2(8)
C9 - N2	1.28(1)	C8 - S2 - C9	88.5(5)
N2 - C7	1.37(1)	C7 - N2 - C9	112.5(9)
C9 - C10	1.41(1)	N2 - C9 - C10	126(1)
C10 - C11	1.40(1)	N2 - C9 - S2	114.2(8)
C11 - S3	1.70(1)	S2 - C9 - C10	119.2(9)
S3 - C12	1.73(1)	C9 - C10 - C11	124(1)
C12 - N3	1.27(1)	C9 - C10 - N3	123(1)
N3 - C10	1.38(1)	C11 - C10 - N3	114(1)
C12 - C13	1.49(1)	C10 - C11 - S3	109.1(9)
C13 - C14	1.38(1)	C11 - S3 - C12	90.2(5)
C14 - C15	1.37(1)	C10 - N3 - C12	109.1(9)
C15 - C16	1.38(1)	N3 - C12 - C13	122.2(9)
C16 - C17	1.35(1)	N3 - C12 - S3	114.6(8)
C17 - C18	1.38(1)	S3 - C12 - C13	123.1(8)
C18 - C13	1.37(1)	C12 - C13 - C18	119(1)
		C14 - C13 - C18	120(1)
		C13 - C14 - C15	117(1)
		C14 - C15 - C16	125(1)
		C16 - C17 - C18	124(1)
		C17 - C18 - C13	119(1)
		C5 - N1 - C6 - O1	5(1)
		C5 - N1 - C6 - C7	-175(2)
		O1 - C6 - C7 - C8	-8(1)

in Table 2.

The three independent molecules in an asymmetric unit are related by a pseudo-threefold symmetry of rotation along the crystallographic a - axis (figure 3). As the phenyl and the bithiazole rings are slightly rotated with respect to each other, the molecule is chiral depending on the sense of rotation. The unit cell contains equal number of molecules of optical antipode (space group $P\bar{1}$) and all the three molecules arranged around a pseudo-threefold axis have the same chirality. The 3-aminopropyldimethylsulphonium side chains of the adjacent molecules are anti-parallel to each other as they are related by inversion centres and there is no contact closer than 3.94Å between them. Each iodine atom is close to two sulphur atoms; one from thiazole of

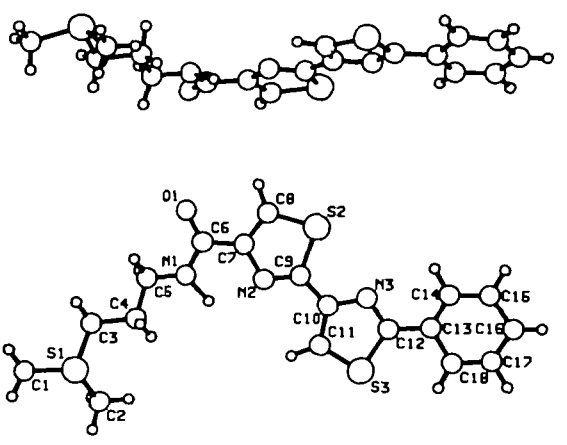

Figure 2. Molecular structure with numbering scheme.

one molecule, and the other from a side chain of another molecule (I1...S3 = 3.918Å, I1...S4 = 3.844Å I2...S1 = 3.830Å, I2...S9 = 3.907Å, I3...S6 = 3.891Å, I3...S7 = 3.834Å).

Bithiazole-containing derivatives whose structures have been solved by X-ray crystallography all appear to exhibit the *trans* orientation of the two rings. These include the isolated bithiazole component of bleomycin, the antibiotic micrococcin P (26), and the derivative described above (25). This orientation is presumably dictated by unfavourable interactions between the C4-hydrogen atom of one thiazole ring and the electrons of the sulphur atom of the other ring. These data suggest that intercalation of the bithaiazole system of bleomycin or its analogues would occur with the rings in a *trans* orientation, in contrast with the *cis* orientation assumed in a recent study (28).

^{1}H NMR spectra with poly (dA-dT) .poly (dA-dT) show (17) that there is a stronger ring current effect of the DNA bases on the phenyl ring (0.41, 0.70 ppm) and on the second thiazole ring (0.86 ppm), but a smaller effect on the first thiazole ring (0.35 ppm) and the side chain hydrogens (0.01 ppm). This indicates that the side chain and the first thiazole ring, to some extent, are further away from the base-pair region of DNA. In the crystal structure, the side chain is fully extended and the long axis of the bithiazole ring and the side chain (~6Å long) form an angle of ca. 95°. The long axis and the exocyclic bond of the phenyl ring form an angle of ca. 135° (figure 2). Therefore it may be possible for the phenyl and the second thiazole ring to

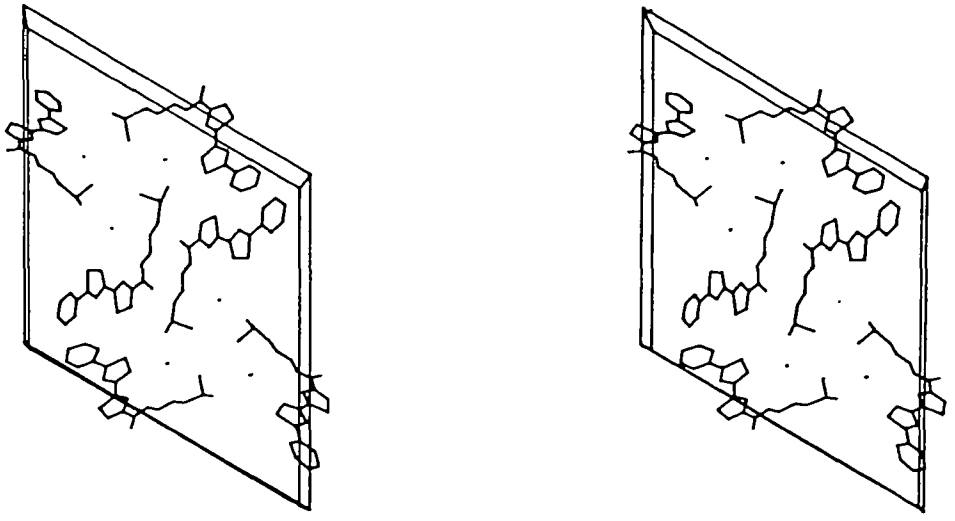

Figure 3. Stereo view of the unit cell along the a axis.

be intercalated between the base pair of duplex DNA and at the same time for the sulphonium cation to interact with the negative phosphate group. Our model-building study shows that such a position is energetically favourable (figure 4). This orientation of the bleomycin derivative forces the first thiazole ring and the side chain out of the base pair region, hence this may explain the weak ring current felt by these protons.

In comparison, the analogous hydrogens of bleomycin itself experience smaller high field shifts in the presence of poly (dA-dT) .poly (dA-dT), with a maximum of approximately 0.3-0.4 ppm (14,17). Similar observations have been made in two studies of bleomycin fragments with poly (dA-dT) .poly (dA-dT) and with self-complementary deoxydinucleoside monophosphates (27,28). In each case, both H5 and H5' resonances of the bithiazole rings exhibit the same shifts. It has been inferred from this data that the bithiazole hydrogens take a *cis* orientation in complexes with deoxydinucleoside monophosphates with both hydrogens pointed in toward the base pair region. In the absence of differential effects, however, it is difficult to formulate a precise model for this orientation from such NMR measurements. It can be shown, for instance, that numerous *cis* and *trans* orientation of the bithiazole system can be found which allow both hydrogens to experience the same shielding effects using the contours of Giessner-Prettre and Pullman (29,30).

Here, we feel that the availability of the crystal structure of the free

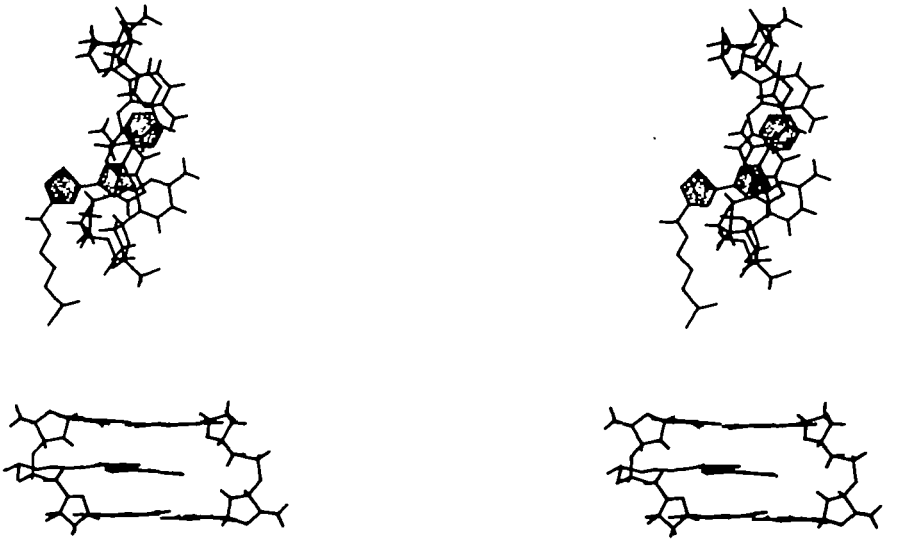

Figure 4. Stereo views of the intercalation model for the bleomycin derivative complexed to d(CpG).

ligand together with the observation of differential effects on hydrogen of the ligand in NMR studies of nucleic acid complexes allows us with some degree of certainty to propose a model for an intercalation complex (figure 4). A more detailed, quantitative study of the interaction of the molecule with models for DNA is currently underway.

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REFERENCES

1 Povirk, L.F., (1982) In Molecular Aspects of Anti-cancer Drug Action, ed. Neidle, S. and Waring, M.J. MacMillan Press, London, in press.
2 Antholine, W.E. and Petering, D.H., (1979) Biochem. Biophys. Res. Commun., 91, 528-533.
3 Hutchinson, F., Povirk, L.F. and Yamamoto, K., (1981) In Molecular Actions and Targets for Cancer Chemotherapeutic Agents, ed. Sartorelli, A.C., Lazo, J.S. and Bertino, J.R. Academic Press, New York.
4 Sugiura, Y. (1978) J. Antibiotics, 31, 1206-1208.
5 Suguira, Y. and Jahizu, K., (1979) J. Inorg. Biochem. 11, 171-180.
6 Suguira, Y. and Kikuchi., (1978) J. Antibiotics, 31, 1310-1312.

7 Oppenheimer, N.J., Rodriguez, L.O. and Hecht, S.M. (1979) Proc. Natl., Acad. Sci. USA, 76, 5616-5620.
8 Murakami, H., Mori, H. and Taira, S., (1973) J. Theor. Biol. 42, 443-460.
9 Iitaka, Y., Nakamura, H., Nakatani, T., Muraoka, Y., Fujii, A., Takita, T. and Umezawa, H. (1978) J. Antibiotics, 31, 1070-1072.
10 Dabrowiak, J.C., Greenaway, F.T. and Grulich, R. (1978) Biochemistry, 17, 4090-4096.
11 Ishizu, K., Murata, S., Myoshi, K., Suguira, Y., Takita, T. and Umezawa, H. (1981) J. Antibiotics, 34, 994-1000.
12 Dabrowiak, J.C. and Tsukayama, M. (1981) J. Amer. Chem. Soc., 103, 7543-7550.
13 Chien, M., Grollman, A.P. and Horwitz, S.B. (1977). Biochemistyr, 16, 3641-3647.
14 Chen, D.M., Sakai, T.T., Glickson, J.D. and Patel, D.J. (1980) Biochem. Biophys. Res. Commun., 92, 197-205.
15 Povirk, L.F., Hogan, M. and Dattagupta, N. (1979) Biochemistry, 18, 96-101.
16 Povirk, L.F., Hogan, M., Dattagupta, N. and Buechner, M. (1981) Biochemistry, 20, 665-671.
17 Booth, T.E., Sakai, T.T. and Glickson, J.D. (1982) Biochemistry, submitted.
18 Asakura, H., Hori, M. and Umezawa, H. (1975) J. Antibiotics, 28, 537-542.
19 Umezawa, H., Takahashi, Y., Fujii, A., Saino, T., Shirai, T. and Takita, T.J. (1973) Antibiotics 26, 117-119.
20 Mirabelli, C.K., Mong, C., Huang, C-H. and Crooke, S.T. (1979) Biochem. Biophys. Res. Commun., 91, 871-877.
21 Mirabelli, C.K., Huang, C-H. and Crooke, S.T. (1980) Cancer Res., 40, 4173-4177.
22 Shieh, H-S., Berman, H.M., Dabrow, M. and Neidle, S. (1980) Nucleic Acids Res., 8, 85-97.
23 Neidle, S. (1981) In Topics in Nucleic Acid Structure, ed. Neidle, S. MacMillan Press, London, p. 177.
24 Berman, H.M. and Neidle, S. (1979) in Stereodynamics of Molecular Systems, ed. Sarma, R.H., Pergamon Press, New York, p. 367.
25 Koyama, G., Nakamura, H., Muraoka, Y., Takita, T., Maeda, K., Umezawa, H. and Iitaka, Y. Tetrahedron Letters, (1968) 4635-4638.
26 James, M.N.G. and Watson, K.J. (1966) J. Chem. Soc. (C), 1361-1371.
27 Sakai, T.T., Riordan, J.M., Booth, T.E. and Glickson, J.D., (1981) J. Med. Chem. 24, 279-285.
28 Lin, S.Y. and Grollman, A.P. (1981) Biochemistry 20, 7589-7598.
29 Giessner-Prettre, C., Pullman, B. (1976) Biochem. Biophys. Res. Commun. 70, 578-581.
30 Giessner-Prettre, C., Pullman, B., Borer, P. Kan, L.-S. and Ts'o, P.O.P. (1976) Biopolymers 15, 2277