Nucleic Acid Binding Drugs. Part 11.† The Structure of 7-Methylellipticine

Reiko Kuroda *
Cancer Research Campaign Biomolecular Structure Research Group, Department of Biophysics, King's College, 26–29 Drury Lane, London WC2B 5RL
Malcolm Sainsbury
School of Chemistry, University of Bath, Claverton Down, Bath BA2 7AY

The molecular geometry of the title compound has been determined by *X*-ray diffraction. The crystal has unit-cell dimensions a = 4.042(2), b = 19.655(4), c = 16.343(2)Å, β = 94.48(2)°, space group $P2_1/n$ and Z = 4. The final R = 0.048 for 806 unique reflections. The ability of various ellipticines to intercalate with DNA was also studied using molecular graphics.

Ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole), E, is a plant alkaloid which shows anti-tumour activity.[1] Its mode of interaction with DNA has been characterised by sedimentation, viscosity, and electric dichroism experiments[2] and shown to involve intercalation of the planar chromophore between DNA base pairs. Crystal structure analysis of an iodo-CpG-ellipticine complex[3] (I-CpG.E), also indicated how intercalation of the drug with DNA might take place. Recently much effort has been devoted to the rational design of ellipticine analogues exhibiting high anti-tumour activity and less cytoxicity, or higher preferential toxicity to cancerous cells. Ellipticine derivatives with various substitutents at 1-, 2-, 5-, 6-, 9-, and 11-positions have been synthesised and their DNA affinity and anti-tumour activity tested.[4–6] From these studies, 9-hydroxyellipticine, 9-OH-E, has been shown to possess high DNA affinity and a lack of cytotoxicity at therapeutic doses. Several other ellipticines have undergone clinical trials: 9-OCH_3-E has been useful in the treatment of human acute myeloid leukemia[7] and 2-CH_3-9-OH-E has shown some effect against advanced human breast cancer.[8] 11-Demethylellipticine showed[9] less DNA affinity and less activity on L1210 mouse leukemia cells than the parent ellipticine and therefore it appears that the presence of a methyl group on the intercalating ring carbon atom, C-11, plays a major role in determining the biological activity of ellipticine and its derivatives. It is interesting that 7-OH and 9-OH derivatives have been found to be metabolites of ellipticine in mice and rats.[10]

One of us has recently developed a direct and versatile synthetic route to various ellipticine derivatives,[11] including the title compound. In this study, the crystal structure of 7-CH_3-E has been determined and the structure is compared with that of other known ellipticine derivatives. Effects of substituents on the ability of ellipticines to intercalate in DNA have also been examined using molecular graphics.

Experimental

Crystal Data.—$C_{18}H_{16}N_2$, M = 260.3, monoclinic, a = 4.042(2), b = 19.655(4), c = 16.343(2) Å, β = 94.48(2)°, U = 1 294(2) Å^3, D_m = 1.34 g cm^{-3}, Z = 4, D_c = 1.336 g cm^{-3}, F(000) = 552, space group $P2_1/n$, Cu-K_α radiation, μ = 0.574 mm^{-1}.

Light yellow, needle-like crystals were grown from ethanol solution. *X*-Ray photographs were taken to determine crystal class. Accurate cell dimensions were obtained by least-squares analysis of 25 θ values measured on an Enraf–Nonius CAD-4 diffractometer. Intensity data were collected on the diffractometer with Ni-filtered Cu-K_α radiation, operated in the ω—2θ scan mode up to θ = 50°. The crystal employed for the *X*-ray diffraction study had the dimensions 0.40 × 0.03 × 0.03 mm. The intensities of three standard reflections were monitored at intervals of 3 600 s and no crystal decomposition was noted. Out of 1 351 unique reflections observed, 806 with $I \geqslant 1.5\sigma(I)$ were used for the refinement. The structure was solved by the direct method with the use of MULTAN 82[12] and refined on F by full-matrix least-squares procedures with anisotropic thermal parameters for non-hydrogen atoms. All the hydrogen atoms were located from difference Fourier syntheses, and their positional and isotropic thermal parameters were refined, with the exception of H(1), H(511), H(711), H(1112), and H(1113) whose thermal parameters were fixed during the refinement. The final difference Fourier map did not show any peaks > 0.20 eÅ^{-3} The final R value was 0.045. A unit weight was assigned to each reflection. The maximum shift/error was 0.03 for non-hydrogen atoms and 0.27 for hydrogen atoms. Empirical absorption[13] and extinction corrections were made. Atomic scattering factors were taken from ref. 14. All calculations were performed on a PDP 11/34A computer using the SDP program systems.[15] Observed and calculated structure amplitudes, and thermal parameters, are listed in Supplementary Publication No. SUP 23966 (8 pp.).‡

Discussion

Final atomic parameters are listed in Table 1. Figure 1 shows a view of the molecule with the numbering scheme employed. Table 2 lists bond lengths and angles. The molecule is highly planar and all the three methyl carbon atoms are on the plane. [Deviations from the plane are 0.008, 0.082, and −0.020 Å for C(51), C(71), and C(111), respectively.] Comparison of the molecular structure with that of parent ellipticine,[16] and 9-methyl and 5-n-butyl-11-dimethyl derivatives[17] reveals virtually no significant differences in the chromophore structure. A weak intermolecular hydrogen bond is observed between N(2) and N(6) [N(2)···H(6) 2.27, N(2)···N(6) 3.14 Å, N(2)···H(6)–N(6) 173°].

To study the ability of ellipticines to intercalate with DNA, empirical energetic calculations were carried out with the aid of molecular graphics using the program MOLEC.[18] The self-complementary dimer of I-CpG in the crystal structure of I-CpG.E[3] was adopted as a DNA model structure. All the hydrogen atom positions in I-CpG, which were not reported in the crystal-structure analysis, were generated and the I-CpG

† Part 10, S. A. Islam and S. Neidle, *Acta Crystallogr., Sect. B.* in the press.

‡ For details of Supplementary Publications see Instructions for Authors in *J. Chem. Soc., Perkin Trans. 2*, 1984, Issue 1.

Table 1. Positional and equivalent isotropic thermal parameters (Å²) with e.s.d.s in parentheses

$$B_{eq} = \frac{4}{3}[a^2B_{11} + b^2B_{22} + c^2B_{33} + acB_{13}\cos\beta]$$

Atom	x	y	z	B_{eq}(Å²)
C(1)	0.718(1)	0.2449(3)	0.4740(4)	3.4(1)
N(2)	0.554(1)	0.1872(2)	0.4601(3)	3.9(1)
C(3)	0.465(2)	0.1544(3)	0.5282(4)	3.9(1)
C(4)	0.532(1)	0.1768(3)	0.6068(3)	2.9(1)
C(4a)	0.709(1)	0.2385(3)	0.6222(3)	2.4(1)
C(5)	0.782(1)	0.2649(3)	0.7021(3)	2.6(1)
C(5a)	0.949(1)	0.3255(3)	0.7074(3)	2.6(1)
N(6)	1.048(1)	0.3624(2)	0.7772(3)	2.8(1)
C(6a)	1.205(1)	0.4219(3)	0.7544(3)	2.6(1)
C(7)	1.333(1)	0.4738(3)	0.8068(3)	3.2(1)
C(8)	1.477(1)	0.5277(3)	0.7683(4)	3.9(1)
C(9)	1.494(2)	0.5313(3)	0.6841(4)	4.2(2)
C(10)	1.368(1)	0.4795(3)	0.6341(3)	3.6(1)
C(10a)	1.217(1)	0.4238(3)	0.6686(3)	2.9(1)
C(10b)	1.050(1)	0.3623(3)	0.6373(3)	2.6(1)
C(11)	0.980(1)	0.3376(3)	0.5593(3)	2.6(1)
C(11a)	0.805(1)	0.2747(3)	0.5519(3)	2.5(1)
C(51)	0.677(1)	0.2287(3)	0.7770(3)	3.5(1)
C(71)	1.315(2)	0.4708(3)	0.8970(4)	4.8(2)
C(111)	1.082(1)	0.3759(3)	0.4857(3)	3.9(1)

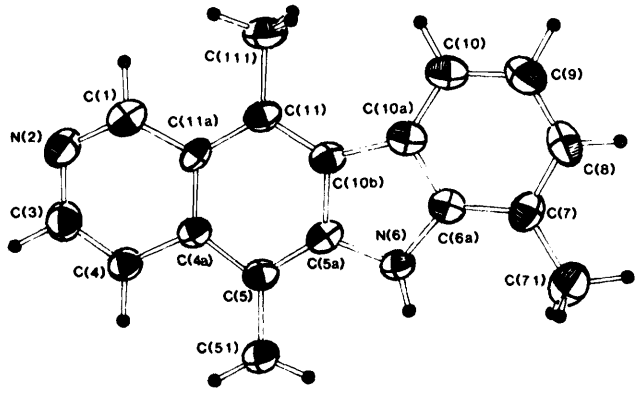

Figure 1. Molecular structure of 7-methylellipticine with the atomic numbering scheme

geometry was held invariant throughout the study. The co-ordinates of ellipticine derivatives were based on those of 7-CH_3-E and appropriate substituent groups were added to or deleted from them. The ellipticine molecules were restricted to rotational and translational movements. The non-bonded energy component was arbitrarily taken as the total interaction energy between the I-CpG dimer and ellipticines in this approximate study; more rigorous analysis would require a total energy minimisation including electrostatic contributions and consideration of flexibility in the receptor site. The results showed that addition of 9-OH or 6-CH_3 groups or deletion of the 11-CH_3 group produced little difference in the interaction energy between I-CpG and the various derivatives. On the other hand, introduction of a 7-CH_3 group produced high energy interactions between the methyl group and O(5′)C(1), C(2′)C(1) and HC(2′)C(1) of I-CpG [C(71)···O(5′)C(1) 2.13 Å, H(711)···O(5′)C(1) 1.41, C(71)···C(2′)C(1) 2.7, H(711)···H(2′)C(1) 1.2 Å], when the substituted ellipticine molecule was placed at the same location as ellipticine in the crystal structure of I-CpG.E. A translation of the drug molecule within the intercalation cavity, particularly along the

Table 2. Bond lengths (Å) and angles (°) for the non-hydrogen atoms with e.s.d.s in parentheses

C(1)–N(2)	1.324(7)	C(6a)–C(7)	1.403(7)
N(2)–C(3)	1.359(7)	C(7)–C(71)	1.483(8)
C(3)–C(4)	1.365(8)	C(7)–C(8)	1.384(8)
C(4)–C(4a)	1.422(7)	C(8)–C(9)	1.386(8)
C(4a)–C(11b)	1.429(7)	C(9)–C(10)	1.377(8)
C(4a)–C(15)	1.414(7)	C(10)–C(10a)	1.392(8)
C(5)–C(51)	1.505(8)	C(10a)–C(10b)	1.458(7)
C(5)–C(5a)	1.368(7)	C(10b)–C(11)	1.374(7)
C(5a)–C(10b)	1.442(7)	C(11)–C(111)	1.504(8)
C(5a)–N(6)	1.385(7)	C(11)–C(11a)	1.424(7)
N(6)–C(6a)	1.395(7)	C(11a)–C(1)	1.420(8)
C(6a)–C(10a)	1.409(7)		

N(2)–C(1)–C(11b)	126.3(6)	C(6a)–C(7)–C(71)	122.3(6)
C(1)–N(2)–C(3)	115.3(5)	C(8)–C(7)–C(71)	122.5(6)
N(2)–C(3)–C(4)	124.9(6)	C(7)–C(8)–C(9)	123.1(6)
C(3)–C(4)–C(4a)	120.1(6)	C(8)–C(9)–C(10)	120.4(6)
C(4)–C(4a)–C(11a)	116.5(5)	C(9)–C(10)–C(10a)	119.6(6)
C(4)–C(4a)–C(5)	122.8(5)	C(6a)–C(10a)–C(10)	118.3(6)
C(5)–C(4a)–C(11a)	120.7(5)	C(6a)–C(10a)–C(10b)	106.0(5)
C(4a)–C(5)–C(5a)	116.3(5)	C(10)–C(10a)–C(10b)	135.7(5)
C(4a)–C(5)–C(51)	121.7(5)	C(5a)–C(10b)–C(10a)	106.9(4)
C(5a)–C(5)–C(51)	121.9(5)	C(5a)–C(10b)–C(11)	120.6(5)
C(5)–C(5a)–N(6)	128.2(5)	C(10a)–C(10b)–C(11)	132.6(5)
C(5)–C(5a)–C(10b)	123.8(5)	C(10b)–C(11)–C(11b)	116.9(5)
N(6)–C(5a)–C(10b)	108.0(5)	C(10b)–C(11)–C(111)	120.9(5)
C(5a)–N(6)–C(6a)	109.2(5)	C(11a)–C(11)–C(111)	122.2(5)
N(6)–C(6a)–C(7)	126.8(5)	C(1)–C(11a)–C(4a)	116.9(5)
N(6)–C(6a)–C(10a)	109.9(5)	C(1)–C(11a)–C(11)	121.3(5)
C(7)–C(6a)–C(10a)	123.3(5)	C(4a)–C(11a)–C(11)	121.8(5)
C(6a)–C(7)–C(8)	115.3(5)		

C(1′)C(1)···C(1′)C(2) direction, however, easily diminished the high energy and a plausible geometry for the intercalation of the drug between the DNA base pairs was then produced. In this situation the degree of overlap between base pairs and the 7-CH_3-substituted drug chromophore was comparable with the case of the parent ellipticine and I-CpG in the crystal structure. Figure 2 compares the intercalation geometry of E and 7-CH_3-E in I-CpG, where the geometry of I-CpG.7-CH_3 represents that of an energy minimum. From inspection of Figure 2, it appears that substitution at any of the 2-,5-,6-,8-,9-,10-, or 11-positions of ellipticine would not be expected strongly to influence intercalation of the chromophore between the base pairs. Biophysical studies also indicate that E, 9-OCH_3-E, 2-CH_3-9-OH-E, 9-OH-E, and 9-NH_2-E can intercalate with DNA.[2,4,19] The results suggest that simple intercalation cannot explain the varied mutagenicity, cytoxicity, and anti-tumour activity of various ellipticines. Recently De Marini *et al.*,[6] from mutagenicity and cytotoxicity tests in mammalian cells and *Salmonella*, showed that E, 9-OCH_3-E, and 9-OH-E appeared to cause frame-shift mutations both by intercalation and covalent binding with DNA, while 9-NH_2-E seemed to exert its mutagenic activity primarily by forming a covalent adduct with DNA. Although there is no strong correlation between the mutagenic activity of the ellipticines in *Salmonella* and their anti-tumour potency in mice, biological data suggest that the ability of E, 9-OCH_3-E, and 9-OH-E to intercalate with DNA, induce frame-shift mutations, and cause cell killing may be the basis for their anti-tumour activity.

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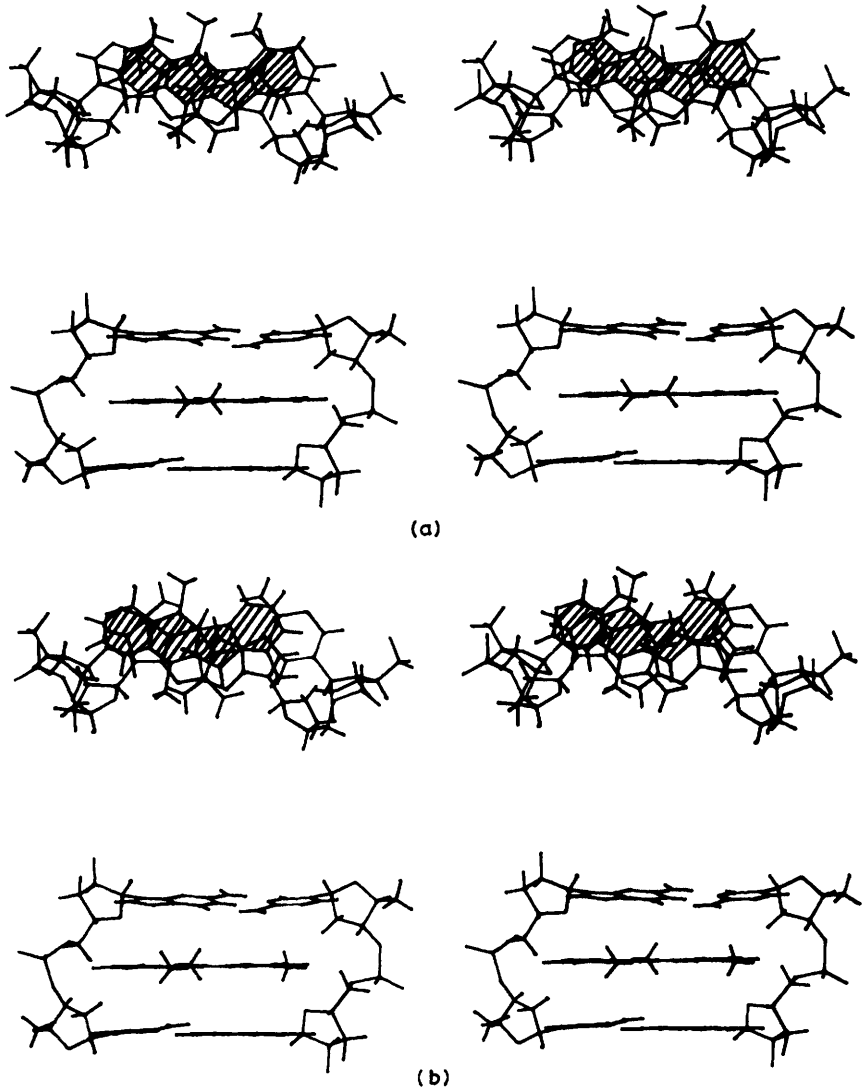

Figure 2. Stereoviews of an intercalation model: (a) ellipticine in I-CpG; (b) 7-methylellipticine in I-CpG

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