Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Structure of 9-amino-[*N*-(2-dimethylamino)propyl]-acridine-4-carboxamide bound to d(CGTACG)$_2$: a comparison of structures of d(CGTACG)$_2$ complexed with intercalators in the presence of cobalt

Adrienne Adams,[a]* J. Mitchell Guss,[a] William A. Denny[b] and Laurence P. G. Wakelin[c]

[a]School of Molecular and Microbial Biosciences, University of Sydney, NSW 2006, Australia, [b]Auckland Cancer Society Research Centre, Faculty of Medicine and Health Science, University of Auckland, Private Bag 92019, Auckland, New Zealand, and [c]School of Medical Sciences, University of New South Wales, NSW 2052, Australia

Correspondence e-mail: a.adams@mmb.usyd.edu.au

Received 13 November 2003
Accepted 19 February 2004

PDB Reference: 9-amino-[*N*-(2-dimethylamino)propyl]-acridine-4-carboxamide–d(CGTACG)$_2$ complex, 1rqy, r1rqysf.

NDB Reference: DD0062.

The structure of the complex formed between 9-amino-[*N*-(2-dimethylamino)propyl]acridine-4-carboxamide and d(CGTACG)$_2$ has been refined to a resolution of 1.55 Å. The complex crystallized in space group *C*222. An asymmetric unit comprises two strands of DNA, one disordered drug molecule, two cobalt(II) ions, two magnesium ions and 32 water molecules. The DNA helices stack in continuous columns, with their four central base pairs adopting a B-like motif. The terminal G·C base pairs engage in different interactions. At one end of the duplex there is a CpG dinucleotide overlap modified by ligand intercalation and terminal cytosine exchange between symmetry-related duplexes. An intercalation complex is formed involving four DNA duplexes, four disordered ligand molecules and two pairs of base tetrads. The other end of the DNA is frayed, with the terminal guanine lying in the minor groove of the next duplex in the column. The structure is stabilized by guanine N7–cobalt(II) coordination. The structure is compared with previously published isomorphous structures of d(CGTACG)$_2$ complexed with intercalators in the presence of cobalt and it is concluded that the formation of this crystal form is primarily determined by DNA–DNA interactions and packing forces, rather than by special interactions between the ligand and the DNA. Given the nature of the ligands found in these complexes, the relevance of the quadruplex structure to the biological activity of those agents, known to be topoisomerase poisons, is questioned.

1. Abbreviations

9-a-p-DACA, 9-amino-[*N*-(2-dimethylamino)propyl]acridine-4-carboxamide; 9-a-b-DACA, 9-amino-[*N*-(2-dimethylamino)-butyl]acridine-4-carboxamide; DACA, *N*-[2-(dimethylamino)-ethyl]acridine-4-carboxamide; 9-amino-DACA, 9-amino-[*N*-(2-dimethylamino)ethyl]acridine-4-carboxamide; bisacridine, macrocyclic bis-9-aminoacridine; phenazine, 9-bromophenazine-4-carboxamide; bis-DACA, bis-[9-aminooctyl-(2-dimethylaminoethyl)acridine-4-carboxamide]; MPD, 2-methyl-2,4-pentanediol.

2. Introduction

DNA-intercalating agents that poison topoisomerase II are important in the treatment of cancer (Denny, 1997; Fortune & Osheroff, 2000; Malonne & Atassi, 1997; Nitiss, 2002) and many of these compounds consist of simple chromophores to which charged side chains are attached that interact with

components of the DNA (Denny, 1997; Malonne & Atassi, 1997). The DNA-intercalated ligand forms a ternary complex with the topoisomerase, thereby trapping it in its cleavable complex, which becomes a cytotoxic lesion leading to double-strand breaks and induction of apoptosis (Fortune & Osheroff, 2000; Li & Liu, 2001; Nitiss, 2002). Structure–activity relationships relating DNA-binding properties to antitumour activity make it clear that the side chains in these agents make specific interactions with elements of the DNA (see, for example, Atwell *et al.*, 1984; Denny *et al.*, 1986, 1987; Rewcastle *et al.*, 1986; Wakelin *et al.*, 1987). We have recently solved the crystal structures of several 9-aminoacridine-4-carboxamides complexed with d(CGTACG)$_2$ (Adams *et al.*, 1999, 2002; Adams, Guss, Collyer, Denny & Wakelin, 2000; Adams, Guss, Collyer, Denny, Prakash *et al.*, 2000; Todd *et al.*, 1999). Four of these structures provide valuable information about the interaction of the chromophore and side chain with the DNA (Adams *et al.*, 1999, 2002; Adams, Guss, Collyer, Denny & Wakelin, 2000; Todd *et al.*, 1999).

In the presence of cobalt and an intercalator, d(CGTACG)$_2$ crystallizes in an unusual quadruplex structure (Adams, Guss, Collyer, Denny, Prakash *et al.*, 2000; Teixeira *et al.*, 2002; Thorpe *et al.*, 2000; Yang *et al.*, 2000) in which four duplexes generate the intercalation cavity from intermolecularly bonded Watson–Crick base pairs. This structure is reminiscent of the multiple-stranded DNA helices seen in recombination and repair processes and the G-quadruplexes formed by telomeric DNA sequences (Neidle & Parkinson, 2003; Ortiz-Lombardia *et al.*, 1999). The intercalating agents in these complexes vary considerably (see Fig. 1 for structures): some have a basic chromophore (Adams, Guss, Collyer, Denny, Prakash *et al.*, 2000), while others are non-basic (Thorpe *et al.*, 2000); some are topoisomerase poisons (Thorpe *et al.*, 2000; Yang *et al.*, 2000), while others are biologically inactive as antitumour agents (Adams, Guss, Collyer, Denny, Prakash *et al.*, 2000) and yet another was designed as a bifunctional intercalating transcription inhibitor (Teixeira *et al.*, 2002; Wakelin *et al.*, 2003). The isomorphous crystal structures were solved independently using MAD phasing in three different laboratories. We present here a new example of the quadruplex structure, that of 9-amino-[*N*-(2-dimethylamino)-propyl]acridine-4-carboxamide bound to d(CGTACG)$_2$ in the presence of cobalt. Although 9-a-p-DACA binds tightly to the alternating copolymer poly-d(GC), it is poorly cytotoxic, has no antitumour activity in the mouse P388 leukaemia model and fails to poison topoisomerase II (Atwell *et al.*, 1984; Denny *et al.*, 1986). The quadruplex structure reported here is closely similar to those published previously (Adams, Guss, Collyer, Denny, Prakash *et al.*, 2000; Teixeira *et al.*, 2002; Thorpe *et al.*, 2000; Yang *et al.*, 2000) and we compare the seven DNA structures in detail in order to gain insight into the molecular determinants of this unusual crystal form, as well as its relevance to understanding the biological properties of topoisomerase II poisons. Given that six of the structures differ only in the nature of the intercalating agent and that their configurations are so similar, it is evident that this crystal form is dominated by the cobalt–guanine interactions, the ability of the terminal C·G base pair to engage in strand exchange and the capacity of the terminal CpG dinucleotides to form intermolecular hydrogen bonds. These features clearly require terminal CpG dinucleotides and are thus nucleotide-sequence dependent. The intercalating agent appears to be a passive element in the structure, serving merely to provide a 'plug' to separate the CpG dinucleotides by 3.4 Å so as to enable the asymmetric unit to pack into the observed crystal motif. If so, since the intercalating agents involved include both topoisomerase poisons and their inactive derivatives, it seems unlikely that the quadruplex structures are relevant to an understanding of the mechanism of topoisomerase poisoning.

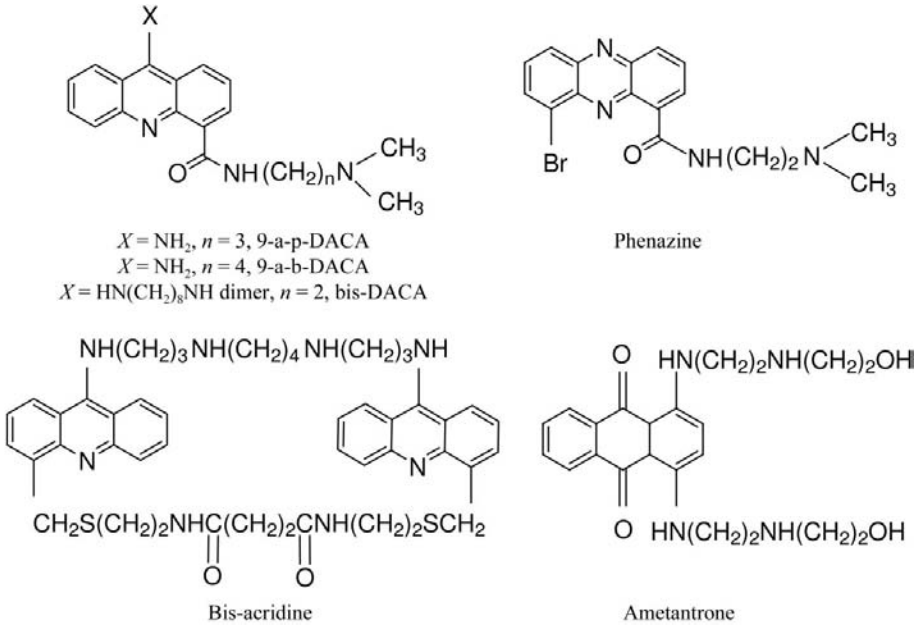

Figure 1
Structure of the compounds crystallized with d(CGATCG)$_2$ in the presence of cobalt(II).

3. Materials and methods

3.1. Crystallization

The HPLC-purified self-complementary deoxyribonucleotide CGTACG was purchased from Oswel DNA Service (University of Southampton, England). 9-a-p-DACA was synthesized as the dihydrochloride salt as previously described (Atwell *et al.*, 1984). The DNA and the ligand were both dissolved in water and stored frozen at 253 K. Crystals were grown at 285 K by vapour diffusion in sitting drops in Cryschem 24-well crystallization plates. Yellow diamond-shaped crystals of approximately 0.3 × 0.2 × 0.1 mm grew in two months from a sitting drop which initially contained 20 m*M* sodium

Table 1
Crystal details, data collection and final refinement parameters.

Values in parentheses are for the last shell (1.61–1.55 Å).

Unit-cell parameters (Å)	a = 28.75, b = 53.12, c = 40.74
Space group	$C222$
No. observations	46070
No. unique reflections	4786
Resolution range (Å)	50–1.55
R_{sym}† (%)	7.7 (26)
Completeness (%)	97.6 (81.7)
$I/\sigma(I)$	18.1 (3.8)
R/R_{free} (%)	22.5/28.2

† $R_{sym} = \sum_{hkl}\sum_i |I_i(hkl) - \overline{I(hkl)}|/\sum_{hkl}\sum_i I_i(hkl)$.

cacodylate buffer pH 6.5, 0.5 m*M* d(CGTACG)$_2$, 1.5 m*M* magnesium acetate, 1 m*M* cobalt chloride, 0.3 m*M* spermine, 1 m*M* 9-a-p-DACA, 4% MPD and was equilibrated against a well containing 35% MPD.

3.2. Data collection, solution and refinement

Crystals were removed from the drop, placed in Riedel de Haen perfluoropolyether RS 3000 oil, mounted in a cryoloop and frozen at 110 K in an N_2 Cryostream. Diffraction intensities were recorded on a Rigaku R-AXIS II image-plate system mounted on a Rigaku RU-200 rotating-anode generator with focusing mirror optics (Cu $K\alpha$, 1.5418 Å) at a crystal-to-detector distance of 65 mm. The data were processed with the *DENZO* and *SCALEPACK* programs (Otwinowski & Minor, 1997), which confirmed that the crystals belonged to the orthorhombic space group $C222$ (Table 1). 8% of the reflections were separated into a reference set in order to monitor R_{free}.

The orthorhombic crystals are isomorphous, R_{deriv} = 0.11, with crystals of 9-a-b-DACA complexed with d(CGTACG)$_2$ (unit-cell parameters a = 28.96, b = 52.60, c = 40.46; Adams, Guss, Collyer, Denny, Prakash *et al.*, 2000; NDB code DD0033). The starting model for the refinement included the DNA components of the 9-a-b-DACA structure, omitting the drug, water molecules and ions. The structure of the 9-a-p-DACA complex was refined using *SHELX*97-2 (Sheldrick, 1997). The drug is disordered and three positions were identified from the $F_o - F_c$ difference density maps and given 0.4, 0.4 and 0.2 occupancy (Fig. 2*a*). There is evidence for a disordered drug side chain in the vicinity of N7 of guanine G8, but it could not be modelled adequately. In this structure we found an additional magnesium ion, which was not found in other similar structures, sitting across a twofold axis. A total of 32 water molecules were observed in the structure. The final R factor was 0.22 and R_{free} was 0.28. Fig. 2(*b*) shows the $3F_o - 2F_c$ electron-density map in the region of the T3·A4 base pair. The coordinates and structure factors have been deposited with NDB code DD0062 and PDB code 1rqy. The structure of the 9-a-p-DACA complex was compared with the six previously published isomorphous intercalated structures of d(CGTACG)$_2$ or d(CG^{5Br}UACG) containing cobalt. The .pdb files of DD0028 [bisacridine–d(CGTACG)$_2$ complex; Yang *et al.*, 2000], DD0029 [ametantrone–d(CGTACG)$_2$ complex; Yang *et al.*, 2000], DD0026 [phenazine–d(CG^{5Br}UACG)$_2$ complex; Thorpe *et al.*, 2000], DD0045 [bis-DACA–d(CGTACG)$_2$ complex; Teixeira *et al.*, 2002], DD0032 [9-a-b-DACA–d(CG^{5Br}UACG)$_2$ complex; Adams, Guss, Collyer, Denny, Prakash *et al.*, 2000] and DD0033 [9-a-b-DACA–d(CGTACG)$_2$; Adams, Guss, Collyer, Denny, Prakash *et al.*, 2000] were obtained from the Nucleic Acid Database (Berman *et al.*, 1992) and all the torsion angles and geometrical properties were analysed using the program *CURVES* (Lavery & Sklenar, 1989). After the seven structures had been transformed into the same asymmetric unit by means of translations and symmetry operations, the structures were aligned with each other in a pairwise fashion to eliminate differences caused by small changes in unit-cell parameters, including all heavy atoms and also treating the bases (side chains) and sugar–phosphate regions (main chains) separately, using the program *LSQKAB* from the *CCP*4 suite of programs (Collaborative Computational Project, Number 4, 1994).

4. Results and discussion

An asymmetric unit of the 9-a-p-DACA–d(CGTACG)$_2$ complex contains two strands of DNA, one disordered drug molecule, 32 water molecules, two cobalt(II) ions and two magnesium ions. No spermine was observed in the electron-density maps. The nucleotides are labelled in the 5′ to 3′ direction from G2 to G6 for strand *A* and C7 to G12 for strand *B*. The DNA strands form duplexes, with the central four base pairs constituting a right-handed B-DNA-like structure

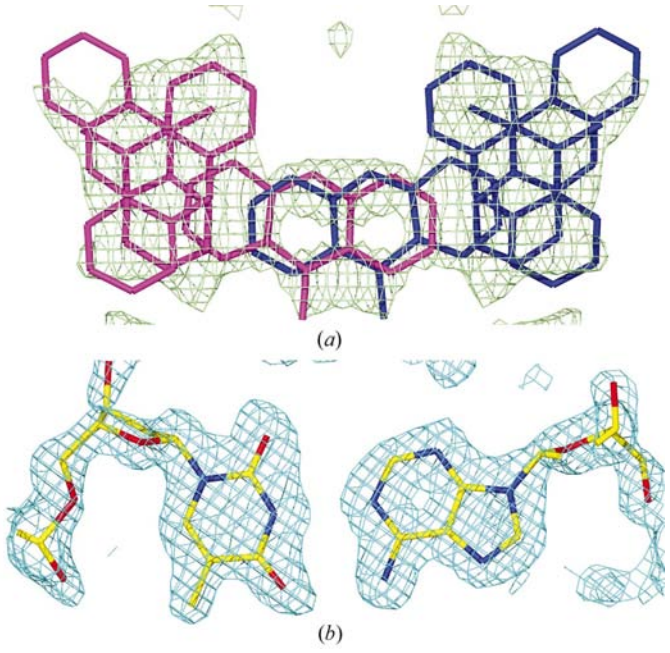

Figure 2
(*a*) $F_o - F_c$ difference electron-density map contoured at 1.5σ in the region of the drug. A twofold axis runs down the page. The three disordered positions for the drug are modelled into the density and coloured blue. The symmetry-related disordered drug molecules are coloured magenta. (*b*) $3F_o - 2F_c$ electron-density map contoured at 2σ in the vicinity of the A4·T9 base pair overlaid on the refined structure.

(Fig. 3). At one end of the duplex, the C5·G8 base pair forms a pseudo-intercalation cavity with a base pair comprising G6 of the same duplex hydrogen-bonded to cytosine C7 of a symmetry-related molecule. At the pseudo-intercalation site, contiguous helices within the same column of duplexes are not stacked end-to-end, but overlap so that their terminal two G·C base pairs interact with each other in their minor grooves. The overlapping base pairs form an almost planar G·C:G·C hydrogen-bonded base quadruplet in which the inter-duplex hydrogen-bonding contacts are between the 2-amino groups and N3 atoms of the guanines. A disordered drug molecule occupies the pseudo-intercalation site. Although electron density for the drug was clearly visible in the $F_o - F_c$ difference density maps, the drug shows a considerable degree of disorder and we have modelled three conformations for the aromatic ring in the binding site such that it occupies most of the available space (Fig. 2*a*). At the other end of the duplex, the terminal guanine G12 is unpaired from cytosine C1 (which is disordered) and the G2·C11 base pair stacks on the G2·C11 base pair of the abutting duplex in the column. The unpaired G12 swings into its own major groove. The backbone torsion angles, sugar puckers and helicoidal properties of this structure (see supplementary material[1]) are very similar to the properties of two previously published structures from our laboratory (Adams, Guss, Collyer, Denny, Prakash *et al.*, 2000) and the four other complexes of intercalators bound to $d(CGTACG)_2$ or $d(CG^{5Br}UACG)_2$ in the presence of cobalt (Teixeira *et al.*, 2002; Thorpe *et al.*, 2000; Yang *et al.*, 2000).

The availability of seven isomorphous structures of ligand–$d(CGATCG)_2$ [or ligand–$d(CG^{5Br}UACG)_2$] complexes, of which six differ only in the nature of the intercalator, provides the opportunity to compare variations in the molecular details of the DNA in these complexes and permits conclusions to be drawn concerning the relevance of these structures to the biological properties of the ligands. Accordingly, we have compared the seven structures after normalizing each to a common asymmetric unit and find that the r.m.s.d. for differences in atomic positions of all the DNA heavy atoms lies between 0.2 and 0.6 Å and that the average r.m.s.d. for the base atoms alone lies between 0.1 and 0.4 Å (Table 2). Fig. 4 illustrates all seven structures after superposition and together with Table 2 confirms their remarkable overall similarity. In addition, pairwise differences between the structures have been calculated separately for the main-chain (sugar and phosphate) and the side-chain (base) atoms for each nucleotide (supplementary Fig. 1 and Tables 17 and 18). The trends observed for the differences from the 9-a-p-DACA complex (supplementary Figs. 1*a* and 1*b*) are replicated when all possible pairs of structures are considered (supplementary Figs. 1*c* and 1*d*), demonstrating that the 9-a-p-DACA complex structure is not an outlier. The deviations are large for the G2 sugar–phosphate region, which might be expected as this nucleotide is attached to the disordered C1 (supplementary Figs. 1*a* and 1*c*). There is an unusually large deviation of the phenazine structure from all other structures at C5 (supplementary Figs. 1*a* and 1*c*). This finding correlates with the unusual α, β and γ torsion angles of the phenazine complex at C5 (see supplementary Tables 1–3). It is possible that the structure of the phenazine complex is truly different in this region or that subjective errors associated with fitting electron density for mobile regions of the structure have been introduced. Ametantrone varies most from all the other structures, as seen in Table 2 and supplementary Figs. 1(*b*) and 1(*d*), having the largest differences both for all atoms and for base atoms alone (Table 2). This finding may be a consequence of

Table 2
R.m.s.d.s (Å) for differences in atomic positions for all heavy atoms (top half of table) and for base atoms (bottom half of table) of the DNA structures superposed in a pairwise fashion.

	9-a-p-DACA	9-a-b-DACA	9-a-b-DACA†	Ametantrone	Bis-acridine	Bis-DACA	Phenazine†
9-a-p-DACA		0.21	0.28	0.58	0.42	0.52	0.50
9-a-b-DACA	0.12		0.26	0.55	0.40	0.51	0.46
9-a-b-DACA†	0.18	0.19		0.53	0.41	0.53	0.47
Ametantrone	0.42	0.41	0.37		0.55	0.55	0.58
Bis-acridine	0.31	0.33	0.32	0.41		0.42	0.51
Bis-DACA	0.27	0.34	0.37	0.35	0.26		0.56
Phenazine†	0.31	0.25	0.25	0.40	0.34	0.31	

† Using $CG^{5Br}UACG$ in place of CGTACG.

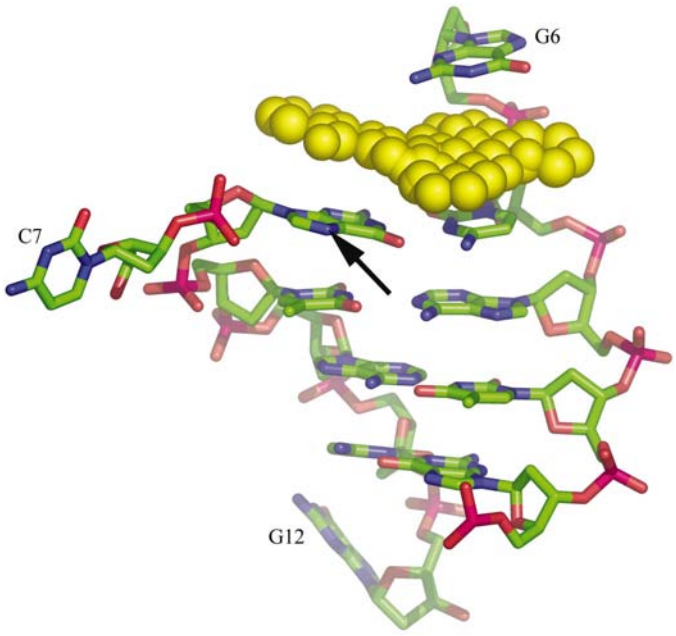

Figure 3
Structure of the 9-a-p-DACA–$d(CGTACG)_2$ complex. The three superposed disordered positions of the drug are shown as yellow space-filling spheres. The arrow indicates the position of N7 of G2 where the side chain of 9-a-p-DACA probably interacts.

[1] Supplementary data have been deposited in the IUCr electronic archive (Reference: HV5010). Details for accessing these data are given at the back of the journal.

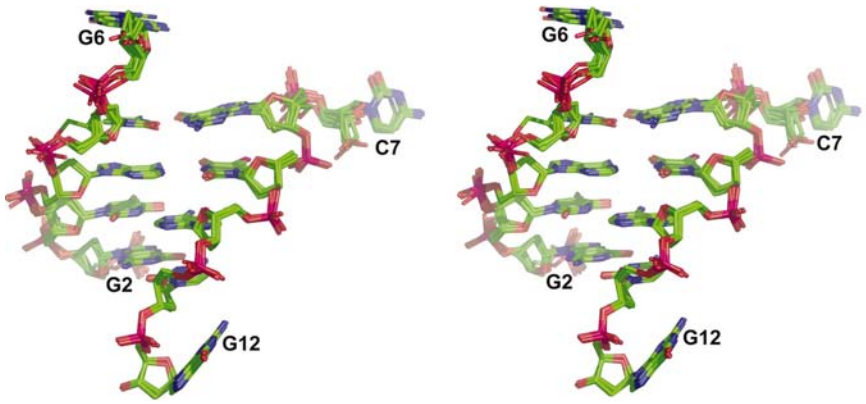

Figure 4
Stereoview of a stick representation of the DNA of the six published structures superposed on the structure of 9-a-p-DACA using CPK colours for the atoms.

the fact that the ametantrone complex differs from the others in having only one drug bound at the double intercalation site. The two bis compounds, bis-DACA and bis-acridine, have large differences at G6 (supplementary Figs. 1*b* and 1*d*). Overall, the greatest differences are observed for the G2, G6, C7 and G8 nucleotides. The last three form the intercalation site in the complexes. The least variable bases are G2, T3, A4, T9, A10 and C11, corresponding to the regular duplex region distal from the intercalation cavity.

From this analysis, it is apparent that the six ligands bind to $d(CGTACG)_2$ in the presence of cobalt in a common structural motif which differs only in minor detail at the G6·C7 pseudo-base pair for the ametantrone and diacridine complexes. Overall, variation in the structures is minimal in the cobalt-binding region and in the central duplex region and is somewhat larger in the quadruplex region, where the regular Watson–Crick structure is disrupted by the base-exchange process. That most variation should occur in the latter part of the structure is perhaps not surprising given that the sugar–phosphate backbone of the terminal cytosine is fully extended in space and is partly held in place by ionic interactions with a magnesium ion. Notwithstanding these minor variations in the position of the pseudo-base pair, we are unable to discern any structural features in the DNA configurations that distinguish one complex from another. From a crystallographic point of view, this is a particularly notable finding given that three of the structures were originally solved *ab initio* by MAD phasing and refined simultaneously in three independent laboratories. The result provides reassuring confirmation of the power and precision of these modern crystallographic methods for analysing unusual non-standard DNA structures. Two laboratories used *CNS* to solve the structures (Adams, Guss, Collyer, Denny, Prakash *et al.*, 2000; Yang *et al.*, 2000) and the other used *SOLVE* (Thorpe *et al.*, 2000); Yang and coworkers using the hexanucleotide $d(CGTA^{5Br}CG)_2$. Although all the refinements were performed using *SHELX*97, Yang and coworkers initially used the simulated-annealing facility in *X-PLOR*. Within the extended data set presented here, the 9-a-p-DACA complex deviates least from the two other structures refined in this laboratory (Adams, Guss, Collyer, Denny, Prakash *et al.*, 2000; Table 2, Fig. 4) and these are the lowest differences observed. We do not find a similar trend for pairs of structures refined in the other two laboratories, *i.e.* differences for ametantrone and bis-acridine and for bis-DACA and phenazine were not lower than when these structures were compared with other structures in the group (Table 2).

In conclusion, it appears that the common structure of the cobalt-containing intercalator $d[CGT(or\ ^{5Br}U)ACG]_2$ complexes is insensitive to the nature of the ligand. It is as though the ligands are passive elements serving merely to provide a 'plug' to separate the CpG dinucleotides by 3.4 Å so as to enable the asymmetric unit to pack into the observed crystal motif. If so, since the intercalating agents involved include both topoisomerase poisons and their inactive derivatives, it seems unlikely that the quadruplex structures are relevant to an understanding of the mechanism of topoisomerase poisoning. In addition, it seems probable that many other intercalators besides those studied crystallographically to date would be capable of forming a similar structure with $d(CGTACG)_2$ in the presence of cobalt and that other DNA sequences having terminal C·G base pairs, which are required for the exchange process and for the hydrogen bonding in the minor groove that establishes the base tetrads, *e.g.* $d(CGATCG)_2$, may also form analogous structures. Lastly, we draw attention to the fact that intercalators evidently have the capacity to destabilize B-DNA in the presence of cobalt ions and to promote the formation of multi-stranded DNA structures. Although we find no evidence for biological significance of this structure in terms of topoisomerase activity, this does not exclude the possibility that this is an important property of intercalating agents that may have relevance to their perturbation of other biological processes involving DNA.

We would like to thank the National Health and Medical Research Council of Australia for financial support.

References

Adams, A., Guss, J. M., Collyer, C. A., Denny, W. A., Prakash, A. S. & Wakelin, L. P. G. (2000). *Mol. Pharmacol.* **58**, 649–658.
Adams, A., Guss, J. M., Collyer, C. A., Denny, W. A. & Wakelin, L. P. G. (1999). *Biochemistry*, **38**, 9221–9233.
Adams, A., Guss, J. M., Collyer, C. A., Denny, W. A. & Wakelin, L. P. G. (2000). *Nucleic Acids Res.* **28**, 4244–4253.
Adams, A., Guss, J. M., Denny, W. A. & Wakelin, L. P. G. (2002). *Nucleic Acids Res.* **30**, 719–725.
Atwell, G. J., Cain, B. F., Baguley, B. C., Finlay, G. J. & Denny, W. A. (1984). *J. Med. Chem.* **27**, 1481–1485.
Berman, H. M., Olson, W. K., Beveridge, D. L., Westbrook, J., Gelbin, A., Demeny, T., Hsieh, S. H., Srinivasan, A. R. & Schneider, B. (1992). *Biophys. J.* **63**, 751–759.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

Denny, W. A. (1997). *Expert Opin. Investig. Drugs*, **6**, 1845–1851.
Denny, W. A., Atwell, G. J., Rewcastle, G. W. & Baguley, B. C. (1987). *J. Med. Chem.* **30**, 658–663.
Denny, W. A., Roos, I. A. G. & Wakelin, L. P. G. (1986). *Anti-Cancer Drug Des.* **1**, 141–147.
Fortune, J. M. & Osheroff, N. (2000). *Prog. Nucleic Acid Res. Mol. Biol.* **64**, 221–253.
Lavery, R. & Sklenar, H. (1989). *J. Biomol. Struct. Dyn.* **6**, 655–667.
Li, T. K. & Liu, L. F. (2001). *Annu. Rev. Pharmacol. Toxicol.* **41**, 53–77.
Malonne, H. & Atassi, G. (1997). *Anti-Cancer Drugs*, **8**, 811–822.
Neidle, S. & Parkinson, G. N. (2003). *Curr. Opin. Struct. Biol.* **13**, 275–283.
Nitiss, J. L. (2002). *Curr. Opin. Investig. Drugs*, **3**, 1512–1516.
Ortiz-Lombardia, M., Gonzalez, A., Eritja, R., Aymami, J., Azorin, F. & Coll, M. (1999). *Nature Struct. Biol.* **6**, 913–917.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Rewcastle, G. W., Atwell, G. J., Chambers, D., Baguley, B. C. & Denny, W. A. (1986). *J. Med. Chem.* **29**, 472–477.
Sheldrick, G. M. (1997). *The SHELX97 Manual.* University of Göttingen, Germany.
Teixeira, S. C. M., Thorpe, J. H., Todd, A. K., Powell, H. R., Adams, A., Wakelin, L. P. G., Denny, W. A. & Cardin, C. J. (2002). *J. Mol. Biol.* **323**, 167–171.
Thorpe, J. H., Hobbs, J. R., Todd, A. K., Denny, W. A., Charlton, P. & Cardin, C. J. (2000). *Biochemistry*, **39**, 15055–15061.
Todd, A. K., Adams, A., Thorpe, J. H., Denny, W. A., Wakelin, L. P. G. & Cardin, C. J. (1999). *J. Med. Chem.* **42**, 536–540.
Wakelin, L. P. G., Atwell, G. J., Rewcastle, G. W. & Denny, W. A. (1987). *J. Med. Chem.* **30**, 855–861.
Wakelin, L. P. G., Bu, X., Eleftheriou, A., Parmar, A., Hayek, C. & Stewart, B. W. (2003). *J. Med. Chem.* **46**, 5790–5802.
Yang, X.-L., Robinson, H., Gao, Y.-G. & Wang, A. H.-J. (2000). *Biochemistry*, **39**, 10950–10957.