Acta Cryst. (1993). D49, 311–317

The Structure of an Idarubicin–d(TGATCA) Complex at High Resolution

BY B. GALLOIS* AND B. LANGLOIS D'ESTAINTOT

Laboratoire de Cristallographie, URA CNRS 144, *Université de Bordeaux* I, 33405 *Talence CEDEX, France*

T. BROWN

Department of Chemistry, University of Edinburgh, West Mains Road, Edinburgh, Scotland

AND W. N. HUNTER†

*Department of Chemistry, University of Manchester, Oxford Road, Manchester M*13 9*PL, England*

(*Received* 1 *July* 1992; *accepted* 23 *September* 1992)

Abstract

The crystal structure of the DNA hexamer d(TGATCA) complexed with the anthracycline antibiotic idarubicin has been determined at 1.6 Å resolution. The asymmetric unit consists of a single hexamer oligonucleotide strand, one drug molecule and 35 water molecules. The complex crystallizes in the tetragonal space group $P4_12_12$, $Z = 8$ with lattice dimensions of $a = b = 28.19$ (3), $c = 52.77$ (4) Å, $V = 41\,935$ Å^3. The structure is isomorphous with a series of hexamer–anthracycline complexes and was solved by molecular replacement. Restrained least-squares methods interspersed with computer-graphics map inspection and model manipulation were used to refine the structure. The R factor is 0.22 for 2032 reflections with $F \geq 3\sigma(F)$ in the resolution range 8.0–1.6 Å. The self-complementary DNA forms a distorted B-DNA double helix with two idarubicin molecules intercalated in the d(TpG) steps of the duplex. The duplex is formed by utilization of a crystallographic twofold axis of symmetry. The idarubicin chromophore is oriented at right angles to the long axis of the DNA base pairs with the anthracycline amino-sugar moiety positioned in the minor groove. Our structure determination allows for comparison with a d(CGATCG)–idarubicin complex recently reported. Despite the sequence alteration at the intercalation step, the structures are very similar. The geometry of the intercalation and the nature of the interactions are conserved irrespective of the DNA sequence involved in the binding.

1. Introduction

The anthracyclines are a family of small organic antibiotics. They consist of a planar aglycone chromophore with an amino-sugar substituent, see Fig. 1. They have been the subject of particular interest over the last 20 years, since the discovery of their potent antitumor activities (Baguley, 1991). Because of their possible role in chemotherapy, many studies have been carried out investigating the biological activities of anthracyclines. It is now well established that their interactions with DNA are of key importance (Valentini, Nicolella, Vannini, Menozzi, Penco & Arcamone, 1985). The anthracyclines bind in a non-covalent manner to DNA and are thus capable of disrupting a variety of cellular processes. The clinical properties of anthracyclines appear to be dependent on their chemical structure and very slight changes in this structure are capable of marked alterations in the clinical efficacy (Brown, 1983; Arcamone & Penco, 1988).

A variety of biophysical methods have been used in the study of anthracycline–DNA interactions (Chaires, 1990). In parallel, X-ray structural investigations have been performed on several anthracycline–DNA complexes. These have served to detail the exact geometry of these interactions and in addition have shown a relationship between structural and biological properties (see, for example, Quigley, Wang, Ughetto, van der Marel, van Boom & Rich, 1980; Moore, Hunter, Langlois d'Estaintot & Kennard, 1989; Wang, Ughetto, Quigley & Rich, 1987; Frederick, Williams, Ughetto, van der Marel, van Boom, Rich & Wang, 1990; Williams, Egli, Gao, Bash, van der Marel, van Boom, Rich & Frederick, 1990; Williams, Frederick, Ughetto & Rich, 1990).

Some anthracycline antibiotics are used clinically despite notable and serious side effects, for example cardiotoxicity. Recent efforts to optimize clinical usefulness whilst diminishing toxicity have led to better tolerated compounds on which attention is now focused. Several X-ray structures of these new drugs complexed with different DNA sequences have already been determined (Gao & Wang, 1991;

* Present address: Department of Structural Chemistry, University of Manchester, Oxford Road, Manchester M13 9PL, England.

† Author to whom correspondence should be addressed.

0907-4449/93/030311-07$06.00 © 1993 International Union of Crystallography

Leonard, Brown & Hunter, 1992; Langlois d'Estaintot, Gallois, Brown & Hunter, 1992).

We present the structure of one of these new antibiotics, idarubicin (Fig. 1) cocrystallized with the DNA hexamer d(TGATCA). Idarubicin differs from the best known anthracycline, daunorubicin, by the replacement of the methoxy group in the 4-position with a proton. Initially synthesized by Arcamone, Bernardi, Giardino, Di Marco, Casazza & Pratesi (1976), its clinical and pharmacological studies have been reported by Daghestani, Arlin, Leyland-Jones, Gee, Kempin, Mertelsman, Budman, Schulman, Baratz, Williams, Clarkson & Young (1985). Results of this structural investigation are presented and compared with those of another idarubicin complex, that with the sequence d(CGATCG) (Gao & Wang, 1991).

2. Chemical synthesis and crystallization

Idarubicin was kindly provided by Farmitalia Carlo Erba Laboratories, Milan, Italy. The hexamer d(TGATCA) was synthesized by phosphoramidite methodology (McBride & Caruthers, 1983) on an Applied Biosystems 381A machine. After cleavage from the resin and deprotection, the oligonucleotide was purified by ion exchange and reverse-phase high-pressure liquid chromatography.

Crystals were grown at 295 K by vapour diffusion from droplets sitting in Corning glass depression plates (McPherson, 1982). Red blocks were obtained within one week, from a solution initially containing 1 m*M* of d(TGATCA), 1.2 m*M* idarubicin, 50 m*M* sodium cacodylate (pH 6.5), 100 m*M* magnesium acetate, 0.5 m*M* spermine hydrochloride plus 10% 2-methyl-2,4-pentanediol (MPD). The solution was equilibrated against a reservoir of 50% aqueous MPD.

3. Data collection and structure refinement

A single crystal of dimensions 0.3 × 0.3 × 0.8 mm was placed in a glass capillary with a droplet of mother liquor and then sealed. Data ($2\theta_{max} = 63°$; $0 \le h \le 19$, $0 \le k \le 19$, $0 \le l \le 35$) were collected on a Rigaku AFC-5 diffractometer with X-rays from an RU200 rotating anode (graphite monochromator, Cu $K\alpha$, λ = 1.54178 Å; 50 kV and 160 mA settings, 0.5 mm focal spot). A 1.0 mm incident collimator was used. The crystal-to-detector distance was 400 mm and a continuously evacuated beam tunnel was in place to reduce absorption by air. The lattice parameters of $a = b = 28.19$ (3), $c = 52.77$ (4) Å, V = 41935 Å^3 were determined from a least-squares fit of 25 reflections with $15 \le 2\theta \le 28°$. The Laue group was identified as $4/mmm$. Reflection conditions of $00l$, $l = 4n$, $h00$, $h = 2n$ identified the space group as either $P4_12_12$ or the enantiomorph $P4_32_12$. Excluding standards, 4578 unique reflections were measured with $\omega/2\theta$ scans using a scan width of $(1.0 + 0.3\tan\theta)°$ at a speed of 16° min^{-1} in ω. Data for which $F/\sigma(F) < 10$ were scanned in triplicate to ensure improved counting statistics for the weaker reflections. Three standard reflections were monitored every 150 measurements throughout data collection. Intensities were corrected for linear decay of 5.5% in addition to corrections for Lorentz and polarization factors. An empirical absorption correction was also applied (North, Phillips & Mathews, 1968) with the transmission range calculated as 0.6–1.0 with an average value of 0.82. The software for data collection and processing was provided by the Molecular Structure Corporation, Texas, USA.

The unit-cell dimensions and symmetry indicated isomorphism with d(TGTACA) complexed with 4′-epiadriamycin in $P4_12_12$ (Leonard *et al.*, 1992) and this structure was used as the starting model for the analysis. Before refinement commenced however, molecular replacement calculations, using the *MERLOT* package (Fitzgerald, 1988) were carried out on this system to check the space-group enantiomer. The translation function was interpretable only in $P4_12_12$, so the coordinates from Leonard *et al.* (1992) were used as the starting model. Water molecules were excluded as was the specific hydroxyl O14 and the methoxy O4 groups of the 4′-epiadriamycin chromophore and the O4′ atom of the sugar moiety known to be no longer in an equatorial but in an axial position.

The structure was refined with the restrained least-squares method of Konnert & Hendrickson (Hendrickson & Konnert, 1981), using the *NUCLSQ* program (Westhof, Dumas & Moras, 1985) implemented on a VAX3100. Electron density ($2F_o - F_c$) and difference ($F_o - F_c$) Fourier maps were calculated using the *CCP*4 suite also on the VAX3100 (SERC Daresbury Laboratory, 1979) and inspected on an Evans & Sutherland graphics workstation. Manipulation of the model, such as the initial swapping of the central base pairs T3–A4 to A3–T4, was per-

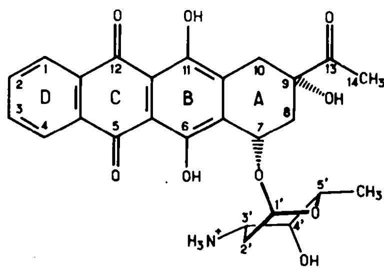

Fig. 1. The molecular formula of idarubicin. The aglycone chromophore consists of three unsaturated (*B*, *C*, *D*) rings and one saturated ring (*A*) covalently bonded to an amino sugar at the 7-position. The functional groups are indicated.

formed with the program *FRODO* (Jones, 1978; Evans, personal communication). Although data were observed to 1.5 Å resolution, intensities were weak between 1.5 and 1.6 Å so that only reflections to 1.6 Å and greater than $3\sigma(F)$ were used in the refinement (*i.e.* 2032 reflections).

Negligible restraints were employed on sugar conformations and at the onset of the refinement, isotropic thermal parameters were fixed to 8, 10 and 12 Å^2 for the bases, the sugars and the phosphate atoms, respectively. Careful interpretation of the electron-density maps was used to guide alterations to the dictionary of restraints. Solvent molecules were gradually added on the criteria that they displayed well defined spherical density in $(F_o - F_c)$ maps and reasonable hydrogen-bonding geometry with neighbouring functional groups. The final refinement of the complex, including 35 water molecules per asymmetric unit, converged at $R = 0.22$. The final model has an r.m.s. deviation in bond lengths from ideal values of 0.02 and 0.03 Å for sugar/base and phosphate groups respectively. Further details relevant to the refinement are presented in Table 1. There were numerous peaks in the difference-density maps that represent disordered solvent sites. We decided not to include these in the final refinement as either their relative densities were considered unsatisfactory or their refined thermal parameters were larger than 60 Å^2. In our analysis we have included significantly fewer solvent molecules than are commonly included for nucleic acid structures of comparable size. Such a rigorous approach clearly influences the quoted R factor. More important than the R factor, from our point of view, is the fit between the final model and the electron density and this is in excellent agreement in all respects (Fig. 2). The atomic coordinates have been deposited with the Brookhaven Data Bank.*

Table 1. *Weighting parameters and agreement factors for the restrained least-squares refinement*

Restraint group and parameters	Standard deviation	R.m.s.	$\Sigma\omega\Delta^2$
Distance restraints (Å)			
Sugar and base bond lengths	0.02	0.02	0.13×10^4
Sugar and base bond angles	0.03	0.04	
Phosphate bond lengths	0.02	0.04	
Phosphate bond angles	0.04	0.05	
99 distances deviate from ideality by more than 2σ			
Planar restraints (Å)			
Deviation from the plane	0.02	0.02	0.10×10^3
Non-bonded contacts (Å)			
Single torsion	0.03	0.13	0.42×10^2
Multiple torsion	0.03	0.15	
Isotropic thermal parameters (Å^2)			
Sugar and base bonds	6.0	3.01	0.26×10^3
Sugar and base angles	6.0	3.78	
Phosphate bonds	6.0	4.44	
Phosphate angles	6.0	4.96	
Unrestrained chiral volumes		0.10	

4. Results and discussion

Structure of the complex

The asymmetric unit consists of one single strand of d(TGATCA), one molecule of idarubicin and 35 ordered water molecules. Two asymmetric units, related by a crystallographic twofold axis, form a right-handed double helix comprising six Watson–Crick base pairs. The duplex is distorted by the intercalation of the nearly planar chromophore at the two TpG steps. In the model, nucleotides are labelled T1–A6 (5′ to 3′ direction) on strand 1, which represents the asymmetric unit, and T7–A12 (5′ to 3′) on strand 2. The idarubicin molecules are labelled *D*13 (asymmetric unit) and *D*14, waters are *W*1 to *W*35. An asterisk will be used to distinguish symmetry-related solvent molecules.

Drawings of the complete duplex and antibiotics are shown in Fig. 3. *D*13 intercalates between C5–G8 and A6–T7; *D*14 between T1–A12 and G2–C11. The long axis of the aglycone is nearly perpendicular to that of the base pairs. The drug spans the two groves of the helix; the three aromatic rings (*B*, *C*, *D*) and the cyclohexene ring (*A*) are well stacked within the DNA duplex. The amino sugar is located in the minor groove of the duplex while the external part of ring *D* protudes in the major groove (Fig. 4).

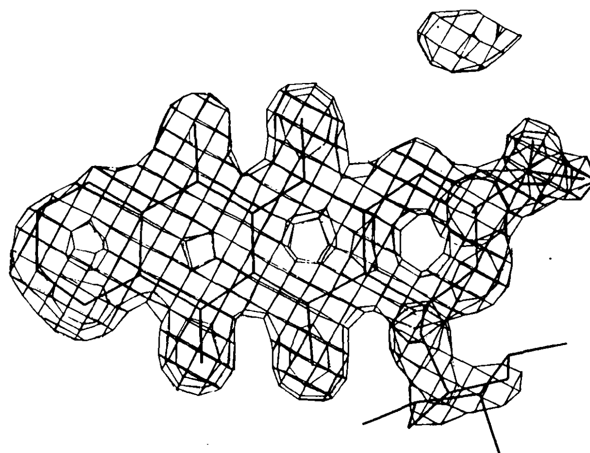

Fig. 2. The aglycone chromophore shown with associated electron density in a $(2F_o - F_c, \alpha_{calc})$ map. The contour level is 1.5 r.m.s. the electron density in the unit cell. The orientation of the aglycone is the same as presented in Fig. 1.

* Atomic coordinates and structure factors have been deposited with the Protein Data Bank, Brookhaven National Laboratory (Reference: 1D67, R1D67SF), and are available in machine-readable form from the Protein Data Bank at Brookhaven. The data have also been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 37072 (as microfiche). Free copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

The overall structure can be considered as B-type DNA. The sugar conformations are mainly C2′-*endo* or similar and, excluding the intercalation steps, the average rise per base pair is approximately 3.6 Å. Table 2 gives the geometrical parameters of the helix, Table 3 lists the sugar–phosphate backbone and glycosidic torsion angles in addition to specific furanose conformations.

The binding of the drug molecules modifies the conformation of the DNA. In the idarubicin–d(CGATCG) complex, Gao & Wang (1991) observed that the DNA backbone torsion angles are significantly rearranged around the drug and that the largest deviations of these angles from those of the uncomplexed B-DNA are associated with the residues involved at the intercalation site. Similar features are seen in our idarubicin–d(TGATCA) complex. These affect mainly the ε and ξ angles of C5 (see Table 3), which provide values (251, 162° respectively) close to those (250, 168°) obtained for the idarubicin–d(CGATCG) complex and differ from the average values (191 and 252°) in the B-DNA dodecamer structure d(CGCGAATTCGCG) (Drew & Dickerson, 1981).

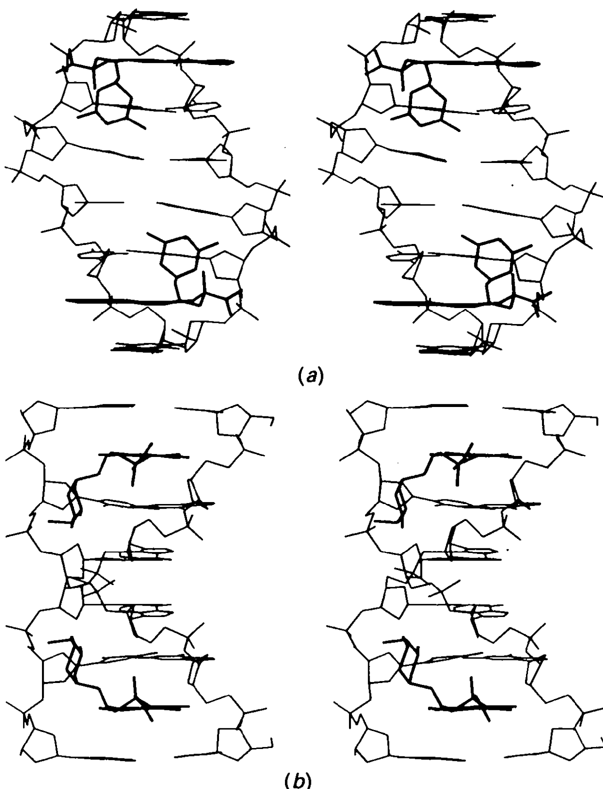

Fig. 3. Stereopairs of the d(TGATCA) duplex complexed with two molecules of idarubicin. The DNA double helix is shown in thin lines, the drugs are drawn in thick lines. (*a*) View along the twofold axis into the major groove. (*b*) View perpendicular to (*a*).

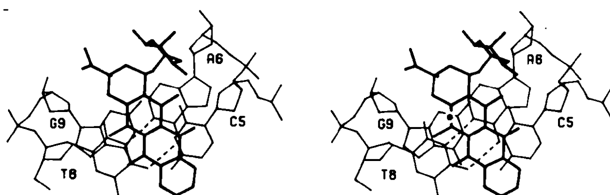

Fig. 4. A stereoview of the projection onto the plane of the idarubicin chromophore with the two surrounding base pairs C–G and A–T of the intercalation site. The drug and the DNA are drawn with thick and thin bonds respectively. The A–T base-pairing hydrogen bonds are shown with dashed lines. The A–T base pair is closer to the viewer, the C–G base pair is further away.

Table 2. *Geometrical parameters of base-pair steps and base pairs*

Values are calculated with the program *NEWHELIX* using the 1989 Cambridge Nomenclature Convention (Diekmann, 1989).

Base pair		Step	Roll (°)	Slide (Å)	Twist (°)	Rise (Å)	Tilt (°)	Propeller twist (°)	Buckle (°)
T1	A12							0.1	−8.8
		1	−0.3	−2.05	35.4	5.3	−0.2		
G2	C11							1.5	15.5
		2	−0.7	0.75	29.9	3.4	−3.6		
A3	T10							3.7	10.0
		3	0.8	3.31	34.3	3.6	0.0		
T4	A9							3.7	−10.0
Average			−0.3	0.14	33.0	4.21	0.00	1.8	0.0

Table 3. *Backbone and glycosyl torsion angles* (°) *and sugar conformations*

Torsion angles along the backbone of the oligonucleotide are defined as $P \overset{\alpha}{—} O5' \overset{\beta}{—} C5' \overset{\gamma}{—} C4' \overset{\delta}{—} C3' \overset{\varepsilon}{—} O3' \overset{\xi}{—} P$ and χ is the glycosyl angle.

	α	β	γ	δ	ε	ξ	χ	p	Furanose conformation
T1	—	—	71	119	240	276	207	132	C1′-*exo*
G2	304	172	41	140	220	176	290	158	C2′-*endo*
A3	307	130	52	119	187	269	245	152	C2′-*endo*
T4	22	180	78	116	200	277	242	131	C2′-*endo*
C5	289	165	45	139	251	162	299	166	C1′-*exo*
A6	297	177	47	149	—	—	283	189	C3′-*exo*
Average	294	165	56	130	220	232	261	155	

At the intercalation steps, the idarubicin molecules unwind the DNA, decreasing the helical twist between the G2–C11 and A3–T10 base pairs by nearly 6° from the B-DNA standard value of 36°. Moreover, the inner G2–C11 and C5–G8 base pairs in the intercalated TpG steps are significantly buckled (15.5°), while the T1–A12 and A6–T7 base pairs present a smaller buckle angle (−8.8°).

These distortions may be explained by differences in the tight fit of the drug–DNA complexes. These interactions will be described in more detail below. Nevertheless, at this juncture let us point out that:

(i) The C—G base pairs are in close contact not only with the amino sugar of the drug but also with both the O9 hydroxyl and the O7 atoms of the chromophore. This is clearly shown in Fig. 5 and

highlighted by several hydrogen-bond interactions formed between N3′---O4′(C5) (at a distance of 2.95 Å), N3′---O2(C5) (2.78 Å), O7---N2(G8) (3.06 Å), O9---N2(G8) (3.12 Å) and O9---N3(G8) (2.79 Å).

(ii) On the other hand, the T—A base pairs, which are also involved in the end-to-end crystal packing, appear to be located further away from the drug. The drug–DNA contacts are less numerous although possible dipole–dipole interactions may involve O11···O2(T7) (3.30 Å) and O11···N3(T7) (3.31 Å).

The conformation of idarubicin

The anthracycline antibiotic idarubicin adopts a very similar conformation in both of the complexes formed with d(TGTACA) and in d(CGATCG).

When defining the least-squares plane of ring *A* by the atoms C7, C8, C10, C19 and C20, the C9 atom is observed to show the greatest deviation. The distances out of the plane are 0.69 Å in the complex with d(TGATCA) and 0.57 Å in the corresponding d(CGATCG) complex. As already mentioned by Gao & Wang (1991), this conformation contrasts with that observed for the anthracycline carminomycin in a free state, *i.e.* not complexed to DNA. Carminomycin is very similar to idarubicin, with the same bond types on the aglycone chromophore so that, in an identical environment, its conformation may be considered as equivalent to that of idarubicin. Since the structure of idarubicin in the free state has not yet been determined carminomycin provides the best reference structure. In free carminomycin, ring *A* adopts a twist-chair conformation, with both the C8 and C9 atoms significantly out of the best plane (Van Dreele & Einck, 1977).

The torsion angles around the glycosyl linkage (C8—C7—O7—C1′ and C7—O7—C1′—C2′) are similar in the two idarubicin complexes. The values, 88/142° and 85/146° for d(TGATCA) and d(CGATCG) complexes strongly differ from those (117/167°) of free carminomycin. These angular modifications must be driven not only by the alteration in the *A*-ring conformation, but also by the

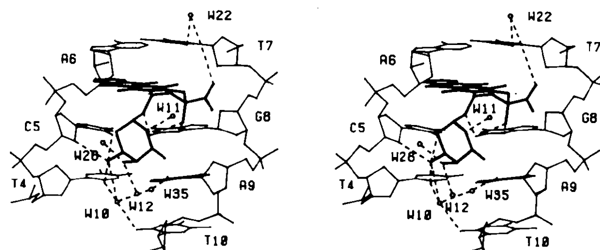

Fig. 5. A stereoview from the minor groove side of the duplex showing the hydrogen-bonding interactions (dashed lines) of idarubicin with both the d(TGATCA) sequence and the water molecules.

Table 4. *Possible hydrogen bonds formed between water molecules and idarubicin*

Drug atom	Water molecule	Distance (Å)	Angle (°)	To third atom
O13	*W*22*	3.23	144	C13 (drug)
O4′	*W*26	2.45	143	C4′ (drug)
O4′	*W*12	3.13	111	C4′ (drug)
N3′	*W*10	3.05	102	C3′ (drug)
O5′	*W*11	3.02	113	C5′ (drug)

* Symmetry operation: $-y, -x, \frac{1}{2}-z$. *W*22* is also bound to O2(T1) and the O13—*W*22*—O2(T1) angle is 99°.

Table 5. *Hydrogen-bonding contacts between DNA and the first shell of water molecules*

Water molecule	DNA atom	Distance (Å)	Angle (°)	To third atom
*W*18	O5′(T1)	3.33	116	C5′(T1)
*W*13	O5′(T1)	2.43	121	C5′(T1)
*W*22	O2(T1)	2.74	155	C2(T1)
*W*15	O4(T1)	2.67	144	C4(T1)
*W*32	O1P(G2)	2.94	134	P(G2)
*W*6	O2P(G2)	2.66	99	P(G2)
*W*7*	O2P(G2)	2.90	142	P(G2)
*W*6	O5′(G2)	2.72	152	C5′(G2)
*W*2	N7(G2)	2.47	112	C8(G2)
*W*5	O1P(A3)	3.41	149	P(A3)
*W*28	O2P(A3)	2.87	143	P(A3)
*W*29	O2P(A3)	2.99	118	P(A3)
*W*35	N3(A3)	2.60	109	C2(A3)
*W*31	N6(A3)	3.39	154	C6(A3)
*W*3	N7(A3)	2.69	123	C8(A3)
*W*7	O3′(A3)	3.06	137	C3′(A3)
*W*1	O1P(T4)	3.26	114	P(T4)
*W*23	O1P(T4)	2.97	138	P(T4)
*W*27	O2P(T4)	3.12	134	P(T4)
*W*7	O2P(T4)	2.98	106	P(T4)
*W*35	O4′(T4)	2.54	110	C4′(T4)
*W*10	O2(T4)	3.18	159	C2(T4)
*W*10†	O2(T4)	2.88	123	C2(T4)
*W*33	O1P(C5)	3.10	156	P(C5)
*W*17	O2P(C5)	2.85	157	P(C5)
*W*4	N4(C5)	2.93	113	C4(C5)
*W*19	O1P(A6)	2.81	107	P(A6)
*W*34	O2P(A6)	2.58	157	P(A6)
*W*19	O5′(A6)	3.28	151	C5′(A6)
*W*14	N3(A6)	3.07	111	C2(A6)
*W*20	N6(A6)	2.58	137	C6(A6)
*W*30	N7(A6)	3.09	119	C8(A6)
*W*8	O3′(A6)	2.71	130	C3′(A6)
*W*24	O3′(A6)	2.92	109	C3′(A6)

* Symmetry operation: $x - \frac{1}{2}, \frac{3}{2} - y, \frac{3}{4} - z$.
† Symmetry operation: $-y, -x, \frac{1}{2} - z$.

Table 6. *Hydrogen bonds formed between idarubicin and DNA*

Drug atom	DNA atom	Distance (Å)
O7	N2(G9)	3.06
O9	N2(G9)	3.12
	N3(G9)	2.79
N3′	O4′(C5)	2.95
	O2(T4)	2.78

hydrogen-bonding interactions between idarubicin and DNA, which are very similar in both complexes, as will now be described.

Hydrogen-bonding interactions

Direct hydrogen-bonding contacts between DNA, idarubicin and solvent molecules are listed in Tables 4, 5 and 6. Direct hydrogen bonds between the

idarubicin molecule and DNA are observed in the minor groove of the duplex (Fig. 5).

The displacement of C9 from the mean plane of ring *A* implies an axial position to the O9 hydroxyl atom. O9 is within hydrogen-bonding distance of two nitrogen atoms (N2 and N3) of G8. In this configuration, O9 simultaneously accepts a proton in a hydrogen bond from N2 and donates a proton in a hydrogen bond to N3. However, the N2—O9—N3 bonding angle is very acute (45°). It is possible, as pointed out by Leonard *et al.* (1992), that in this geometrical arrangement only one hydrogen bond exists, *i.e.* between the aglycone O9 and N3.

The N3′ atom of the amino sugar is in close contact with three acceptor groups in the minor groove. It forms two hydrogen bonds with O4′(C5) and O2(T4) and possibly a third one with O2(C5). The N2 amino group of G9 donates a hydrogen bond to the O7 atom of ring *A*.

Two indirect hydrogen bonds also link the drug to the DNA involving, in each case, mediation by a water molecule. The C13 carbonyl oxygen is bonded to O2 of the T7 terminal residue *via* *W*22 [O13—*W*22—O2(T7) angle is 99°] helping to stabilize the formation of the complex. The N3′ atom of the amino sugar is bridged to the O2 atom of T10 through *W*10 [N3′—*W*10—O2(T10) angle is 106°]. Such solvent-mediated hydrogen-bonding interactions are a common feature of many DNA–anthracycline complexes. They have been observed previously in daunomycin–d(CGTACG) (Wang *et al.*, 1987), adriamycin and daunomycin–d(CGATCG) or daunomycin–d(CGTACG) (Frederick *et al.*, 1990), 4′-epiadriamycin–d(CGATCG) (Williams *et al.*, 1990) and 4′-epiadriamycin–d(TGATCA) (Langlois d'Estaintot *et al.*, 1992).

Nevertheless, in our complex, as in the daunomycin- and adriamycin–DNA complexes, the *W*10 molecule is not interacting with the 4′-hydroxyl group of the amino sugar. This contrasts with 4′-epiadriamycin–DNA complexes, and is a direct result of the inversion of the stereochemistry at the 4′-position of the amino sugar in the 4′-epiadriamycin complexes. In idarubicin, the O4′ atom, no longer aligned in an axial position, is hydrogen bonded to two different water molecules (*W*26 and *W*12) and not involved in any direct hydrogen bonding with the DNA.

5. Concluding remarks

The structure determination of the complex formed by the anthracycline antibiotic idarubicin and d(TGATCA) allows for a direct comparison with the complex formed with another hexanucleotide, d(CGATCG) and also for a general comparison with a series of anthracycline–DNA complexes. The overall results are in good agreement with previous studies. The geometry of the idarubicin–d(TGATCA) complex follows the general rules of intercalation with the drug positioned at a right angle to the long axis of the base pairs and the amino sugar being located in the minor groove. The base pairs deform (buckle and twist) to wrap around the intercalator and thus maximize van der Waals contacts. There are direct hydrogen bonds formed between idarubicin and DNA in the minor groove, in a similar pattern to that usually observed, the stabilization of the insertion of a drug aglycone chromophore being reinforced by a water-mediated hydrogen bond involving *W*22.

Of particular note is the very close similarity observed between the idarubicin–d(TGTACA) complex with the idarubicin–d(CGATCG) structure. Specific similarities of note are the rearrangement of the DNA backbone in the vicinity of the drug, the similar conformations of the idarubicin molecules and also the distortions in the adjacent base pairs at the intercalation site. Buckle angle values of the C–G inner base pairs are comparable [16 and 17° in d(TGATCA) and d(CGATCG), respectively], the only slight difference arising from those of the external T–A or C–G base pairs (9, 14°).

These similarities suggest to us that whatever the considered insertion sequence, the idarubicin stacks within the DNA in the same position as daunomycin does. This implies that the missing C-methoxy group (at the 4-position of ring *D*) in idarubicin does not significantly alter the stacking interactions between the antibiotic and the DNA bases. Furthermore the crystal structures suggest that idarubicin displays an inability to differentiate between intercalation at TpG or CpG sites. Whereas the adjacent bases to the intercalation site may play a role in determining the energetics of binding and perhaps a small preference if a long stretch of DNA is considered the flexibility inherent in the amino sugar and the glycosidic linkage will allow a variety of sequences to act as binding sites for this antibiotic. To tailor anthracycline specificity would perhaps require chemical modification to reduce flexibility.

BG is grateful to the Royal Society for a twelve month fellowship in the European Science Exchange Program, BLd'E thanks EMBO for a short term fellowship. This research is supported by the Wellcome Trust, the Science and Engineering Research Council (UK) and the Royal Society. We acknowledge Farmitalia for a gift of idarubicin, P. R. Evans for his version of *FRODO* and C. Murphy for technical assistance. Finally, we would like to thank Dr G. A. Leonard for providing the coordinates of the complex d(TGTACA)–4′-epiadriamycin and for helpful discussions.

References

ARCAMONE, F., BERNARDI, L., GIARDINO, P., DI MARCO, A., CASAZZA, A. M. & PRATESI, G. (1976). *Cancer Treat. Rep.* **60**, 829–834.

ARCAMONE, F. & PENCO, S. (1988). *Anthracycline and Anthracenedione Based Anticancer Agents*, edited by J. W. LOWN, pp. 1–53. New York: Elsevier.

BAGULEY, B. C. (1991). *Anticancer Drug Des.* **6**, 1–35.

BROWN, J. R. (1983). *Molecular Aspects of Anticancer Drug Action*, edited by S. NEIDLE & M. J. WARING, pp. 57–92. London: Macmillan.

CHAIRES, J. B. (1990). *Biophys. Chem.* **35**, 191–202.

DAGHESTANI, A. N., ARLIN, Z. A., LEYLAND-JONES, B., GEE, T. S., KEMPIN, S. J., MERTELSMAN, R., BUDMAN, D., SCHULMAN, P., BARATZ, R., WILLIAMS, L., CLARKSON, B. D. & YOUNG, C. W. (1985). *Cancer Res.* **45**, 1408–1412.

DIEKMANN, S. (1989). *J. Mol. Biol.* **205**, 787–791.

DREW, H. R. & DICKERSON, R. E. (1981). *J. Mol. Biol.* **151**, 535–556.

FITZGERALD, P. M. D. (1988). *J. Appl. Cryst.* **21**, 273–378.

FREDERICK, C. A., WILLIAMS, L. D., UGHETTO, G., VAN DER MAREL, G. A., VAN BOOM, J. H., RICH, A. & WANG, A. H.-J. (1990). *Biochemistry*, **29**, 2538–2549.

GAO, Y. G. & WANG, A. H.-J. (1991). *Anticancer Drug Des.* **6**, 137–149.

HENDRICKSON, W. A. & KONNERT, J. H. (1981). *Biomolecular Structure Conformation, Function and Evolution*, Vol. 1, edited by R. SRINIVASAN, pp. 43–57. Oxford: Pergamon.

JONES, T. A. (1978). *J. Appl. Cryst.* **11**, 268–272.

LANGLOIS D'ESTAINTOT, B., GALLOIS, B., BROWN, T. & HUNTER, W. N. (1992). *Nucleic Acids Res.* **20**, 3561–3566.

LEONARD, G. A., BROWN, T. & HUNTER, W. N. (1992). *Eur. J. Biochem.* **204**, 69–74.

MCBRIDE, L. J. & CARUTHERS, M. H. (1983). *Tetrahedron Lett.* **24**, 245–248.

MCPHERSON, A. (1982). *Preparation and Analysis of Protein Crystals.* San Francisco: Wiley.

MOORE, M. H., HUNTER, W. N., LANGLOIS D'ESTAINTOT, B. & KENNARD, O. (1989). *J. Mol. Biol.* **206**, 693–705.

NORTH, A. C. T., PHILLIPS, D. C. & MATHEWS, F. S. (1968). *Acta Cryst.* A**24**, 580–584.

QUIGLEY, G. J., WANG, A. H.-J., UGHETTO, G., VAN DER MAREL, G. A., VAN BOOM, J. H. & RICH, A. (1980). *Proc. Natl Acad. Sci. USA*, **77**, 204–7208.

SERC Daresbury Laboratory (1979). *CCP4. A Suite of Programs for Protein Crystallography.* SERC Daresbury Laboratory, Warrington WA4 4AD, England.

VALENTINI, L., NICOLELLA, V., VANNINI, E., MENOZZI, M., PENCO, S. & ARCAMONE, F. (1985). *Farmaco (Pavia)*, **40**, 377–383.

VAN DREELE, R. B. & EINCK, J. J. (1977). *Acta Cryst.* B**33**, 3282–3288.

WANG, A. H.-J., UGHETTO, G., QUIGLEY, G. J. & RICH, A. (1987). *Biochemistry*, **26**, 1152–1163.

WESTHOF, E., DUMAS, P. & MORAS, D. (1985). *J. Mol. Biol.* **184**, 119–145.

WILLIAMS, L. D., EGLI, M., GAO, Q., BASH, P., VAN DER MAREL, G. A., VAN BOOM, J. H., RICH, A. & FREDERICK, C. A. (1990). *Proc. Natl Acad. Sci. USA*, **87**, 2225–2229.

WILLIAMS, L. D., FREDERICK, C. A., UGHETTO, G. & RICH, A. (1990). *Nucleic Acids Res.* **18**, 5533–5541.