Structure of the Altromycin B (N7-Guanine)–DNA Adduct. A Proposed Prototypic DNA Adduct Structure for the Pluramycin Antitumor Antibiotics†

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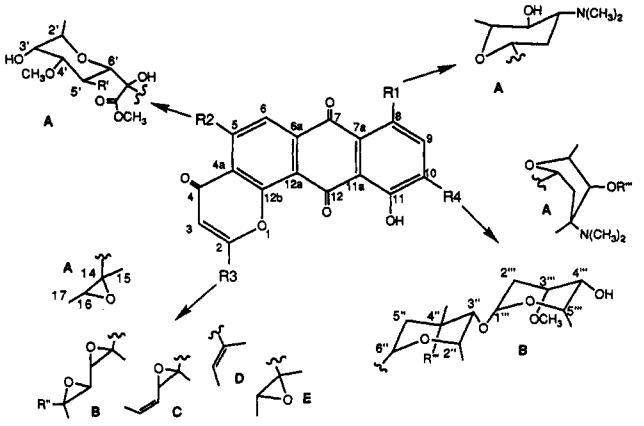
ABSTRACT: Altromycin B belongs to the pluramycin family of antitumor antibiotics, which also includes kidamycin, hedamycin, pluramycin, neopluramycin, DC92-B, and rubiflavin A. These potent antitumor compounds react with DNA in as yet imprecisely determined ways. In the present investigation, we have used gel electrophoresis methods in combination with nuclear magnetic resonance and mass spectrometry to determine the structure of the altromycin B–DNA adduct. High-resolution gel electrophoresis demonstrated that guanine was the reactive base, and N7 was implicated from experiments in which N^7-deazaguanine was used in place of guanine in a strand breakage assay. Experiments using supercoiled DNA demonstrated that altromycin B and related drugs intercalated into DNA, which implicated this as a common mechanism for binding of the pluramycin antibiotics to DNA. The altromycin B–guanine adduct was isolated from calf thymus DNA after thermal depurination of the alkylated DNA. Mass spectrometry confirmed that altromycin alkylated DNA through guanine, and ^{1}H- and ^{13}C-NMR was used to confirm the covalent linkage sites between altromycin B and guanine. On the basis of these results, we propose that altromycin B first intercalates into DNA via a threading mechanism, reminiscent of nogalamycin, to insert the disaccharide into the minor groove and position the epoxide in the major groove in proximity to N7 of guanine. Nucleophilic attack from N7 of guanine leads to an acid-catalyzed opening of the epoxide, resulting in the altromycin B–DNA adduct. On the basis of these results, a general mechanism for the interaction of the pluramycin family of antibiotics with DNA is proposed.

Altromycin B is a new antitumor antibiotic produced by an actinomycete (strain AB 1246E-26) and was discovered through an antitumor screening program at Abbott Laboratories, Chicago, IL (Jackson et al., 1990; Brill et al., 1990). This antibiotic and its structural analogs are members of the family of pluramycin antibiotics (4*H*-anthra[1,2-*b*]pyran antibiotics) that also includes pluramycin A, kidamycin, hedamycin, and rubiflavin B [reviewed by Sequin (1986)]. The structures of altromycin B and related antibiotics are summarized in Figure 1. These compounds show antitumor and antimicrobial activity (Sequin, 1986; Jackson et al., 1990) and react in as yet imprecisely determined ways with DNA.

Altromycin B is one of a group of structurally related compounds (see Figure 1) that show *in vitro* activity against human and murine cell lines and *in vivo* activity in P388 leukemia, M5076 ovarian sarcoma, Lewis lung carcinoma, and human LS174T colon cancer (McAlpine et al., 1992). In initial mechanism of action studies (McAlpine et al., 1992), altromycin B was shown to preferentially inhibit RNA and DNA synthesis relative to protein synthesis, and the inhibition of cell growth could be prevented by coaddition of calf thymus DNA. While intercalation was not detected using an unwinding assay, both grooves were implicated in the binding of altromycin B to DNA.

In the present study, we have used gel electrophoresis methods to show that altromycin B covalently modifies N7 of guanine. ^{1}H- and ^{13}C-NMR have been used to confirm the guanine alkylation site and show that the epoxide is the DNA reactive group on altromycin B. On the basis of a DNA unwinding assay, intercalation is demonstrated to be the general mode of precovalent binding. On the basis of these results, a model of the altromycin B (N7-guanine)–DNA adduct is proposed, and this model is extended to provide a



Compound	R1	R2	R3	R4	R'	R''	R'''
Altromycin A	H	A	A	B	OH		$NHCH_3$
Altromycin B	H	A	A	B	OH		$N(CH_3)_2$
Altromycin C	H	A	A	B	H		$NHCH_3$
Altromycin D	H	A	A	B	H		$N(CH_3)_2$
Altromycin H	H	OH	A	B			$N(CH_3)_2$
Altromycin I	H	OH	A	B			$NHCH_3$
Pluramycin	A	CH_3	C	A			Acetyl
Neopluramycin	A	CH_3	D	A			Acetyl
Hedamycin	A	CH_3	B	A		H	H
DC92-B	A	CH_3	B	A		CH_3	H
Kidamycin	A	CH_3	D	A			H
Epoxykidamycin	A	CH_3	E	A			H
Rubiflavin A	A	CH_3	C	A			H

FIGURE 1: Structures of altromycins A, B, C, D, H, and I; pluramycin; neopluramycin; hedamycin; kidamycin; epoxykidamycin (Byrne et al., 1985); rubiflavin A; and DC92-B (Yasuzawa et al., 1990).

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Table I: Sequences of Oligomer DNA Used in This Study

I	5′ CCAACCCGTAATTAGGTGCGG 3′ 3′ TGGGCATTAATCCACGCCGGT 5′
II	5′ GAGCACCGCAAAAACGGATTG 3′ 3′ GTGGCGTTTTTGCCTAACCTC 5′
III	5′ CCACATCCAAG*CATACCCAAT 3′ 3′ GGTGTAGGTTCGTATGGGTTA 5′

general thesis for the interaction of the pluramycin antibiotics with DNA. The relationship of the mechanism of action of the pluramycins to kapuramycin A_3 (Hara et al., 1990) and aflatoxin B_1 (Gopolakrishnam et al., 1990) is also discussed.

MATERIALS AND METHODS

Chemicals and Enzymes. The drugs used in this study were obtained from Abbott Laboratories, Chicago, IL. Electrophoretic reagents [acrylamide, TEMED,[1] ammonium persulfate, and bis(acrylamide)] were purchased from Bio-Rad. T4 polynucleotide kinase and Klenow fragment were from U.S. Biochemical Corp., and wheat germ topoisomerase I was from Promega. [γ-^{32}P]ATP, [α-^{32}P]dCTP, and X-ray film were from ICN.

Preparation of Oligonucleotides. A series of oligonucleotides (Table I) were synthesized on an automated DNA synthesizer (Applied Biosystems 381A) by the phosphoramidite method. The oligomers were then deprotected separately with saturated ammonium hydroxide at 55 °C overnight, dried under vacuum, and redissolved in DDW.

Preparation of Labeled Duplexes. To construct the 5′-^{32}P single end-labeled duplex, one strand of duplex was kinated for 2 h at 37 °C in 25 μL of solution containing 70 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 5 mM dithrothreitol, 30 μCi of [γ-^{32}P]ATP, 10 units of T4 polynucleotide kinase, and 5 μg of DNA. Reaction mixtures were heated at 95 °C for 10 min to inactivate T4 polynucleotide kinase and annealed to the complementary cold strand to generate the duplexes. 3′-^{32}P end-labeling was achieved by a filling-in reaction with the cold duplex, using [α-^{32}P]dCTP and 10 units of Klenow fragment incubated for 1 h at 37 °C in 20 μL of solution containing 70 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, and 5 mM dithiothreitol. Labeled duplexes were electrophoresed on the 8% nondenaturing polyacrylamide gel to purify DNA using the method described previously (Lee et al., 1991).

Drug Binding Reactions and DNA Strand Breakage Assay. Drug binding reactions were carried out at room temperature in a solution containing 10 mM Tris-HCl (pH 7.6), 50 mM NaCl, oligomer duplex, and the indicated amount of drug molecules. Drug binding reactions were stopped by phenol/$CHCl_3$ extraction and ethanol precipitation to remove unbound drug molecules. DNA pellets were dried and redissolved in DDW. For the strand breakage assay, drug-modified DNA samples were heated to 95 °C, with or without piperidine (1 M), for 30 min. DNA samples were mixed with the same volume of alkaline dye (80% formamide, 10 mM NaOH) and then subjected to 20% denaturing gel electrophoresis in parallel with DNA sequencing reactions prepared as described before (Maxam & Gilbert, 1980).

DNA Unwinding Assay Using Topoisomerase I. To test whether the pluramycins unwind DNA upon binding to DNA, 1 μg of sc DNA was reacted with topo I in the presence of drug molecules in 20 μL of solution containing 20 mM Tris-HCl (pH 7.6), 50 mM KCl, and 10 mM NaCl for 1 h at 37 °C. Reactions were stopped by the addition of stop buffer containing 50% glycerol, 10 mM EDTA, and 2% sodium dodecyl sulfate. Reaction mixtures were electrophoresed on 1% agarose gel to separate the topoisomers generated.

Preparation of the Altromycin B–Guanine Adduct. The altromycin B–guanine adduct was prepared by incubating 50 mg of altromycin B with 1 g of calf thymus DNA in a sodium phosphate buffer for 2 days, after which time the mixture was extracted with 1-butanol to remove all the unreacted drug. The remaining altromycin B–DNA adduct was repeatedly heated at 90 °C for 5–10 min, followed by butanol extraction to remove the depurinated product. The butanol phase, containing the altromycin base adduct, was back-extracted with water several times and lyophilized to dryness.

Spectroscopic Experiments. All two-dimensional NMR experiments were performed on a Bruker AMX500 spectrometer at 333 K in a 4:1 deuterated DMSO/benzene solvent system and referenced internally to the solvent DMSO and externally to $^{15}NH_4Cl$, except for the ROESY experiment, which was run in deuterated methanol, referenced internally to methanol, at 300 K. Assignments were accomplished through the execution of two-dimensional ^{1}H–^{13}C correlation, homonuclear TOCSY, NOESY, and ROESY experiments, and ^{1}H–^{13}C and ^{1}H–^{15}N two-dimensional HMBC experiments.

RESULTS

The presence of both a planar 4*H*-anthra[1,2-*b*]pyran-4,7,-12-trione moiety and an epoxide in altromycin B (Figure 1) suggested both intercalation and alkylation as possible mechanisms for interaction with DNA. Experiments using sc DNA to measure intercalative unwinding and high-resolution gel electrophoresis to determine DNA strand breakage induced by base modification were designed to test these ideas.

The 4H-Anthra[1,2-b]pyran Ring System of the Pluramycins Intercalates into DNA. The standard test for intercalation involves an assay using plasmid DNA in combination with topo I, which converts sc DNA (form I) to relaxed DNA (relaxed form I). Form III is linear duplex DNA produced by the introduction of ds breaks into form I DNA. Agarose gel electrophoresis can be used to monitor interconversion between these different forms. If an intercalative drug like ethidium bromide is present, the sc DNA is unwound, and topo I will relax the drug-induced positive superhelical turns. Removal of ethidium bromide leaves sc DNA, which migrates differently in agarose gel electrophoresis than relaxed DNA. The success of this assay depends upon the removal of drug from relaxed DNA after incubation with topo I. If the drug either nicks DNA or remains covalently bound to the DNA, any associated intercalation will remain undetected by the assay. Since we suspected that the epoxide of altromycin B might alkylate DNA, we selected a number of anthrapyran antibiotics, some of which (neopluramycin and kidamycin) lacked this potentially DNA-reactive moiety. The results of this assay using altromycin B, hedamycin, neopluramycin, and kidamycin are shown in Figure 2. In control lane 1, two

[1] Abbreviations: MS, monomeric supercoiled; DS, dimeric supercoiled; MR, monomer relaxed; DR, dimer relaxed; DDW, doubly distilled water; ss, single stranded; ds, double stranded; sc, supercoiled; EtdBr, ethidium bromide; topo I, DNA topoisomerase I; FAB-MS, fast atom bombardment mass spectrometry; DMSO, dimethyl sulfoxide; TOCSY, total correlation spectroscopy; NOE, nuclear Overhauser effect; NOESY, two-dimensional NOE correlated spectroscopy; ROESY, rotating-frame NOE spectroscopy; HMBC, heteronuclear multiple bond correlation; AP, apurinic; TEMED, *N,N,N′,N′*-tetramethylethylenediamine; Tris, tris(hydroxymethyl)aminomethane; ATP, adenosine 5′-triphosphate.

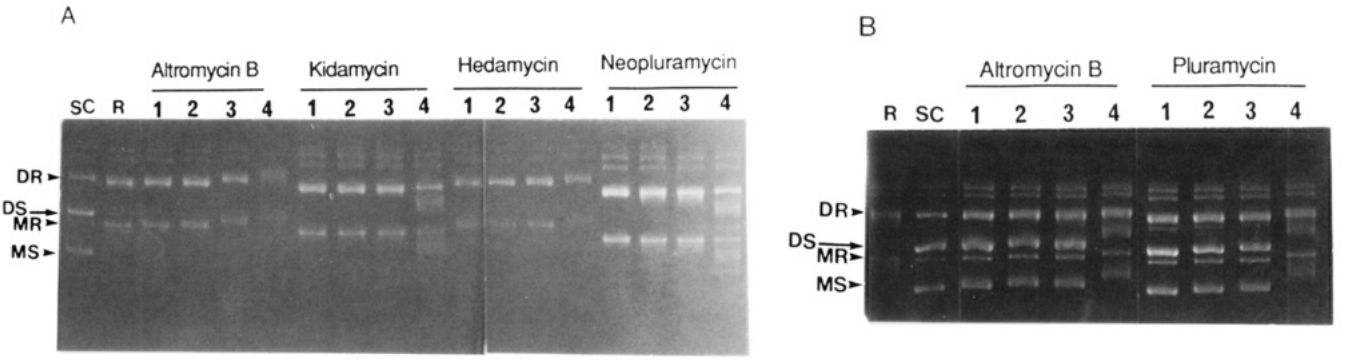

FIGURE 2: (A) Topo I-induced relaxation assay to analyze the unwinding ability of pluramycin antibiotics. SC and R refer to control plasmid DNA pUC19 and the same DNA reacted with topo I, respectively. Lane headings represent the drug molecules used in the experiment. Lanes 1, 2, 3, and 4 contain 35, 70, 150, and 300 ng of drug molecules, respectively. (B) Mobility shift of plasmid DNA pUC19 induced by altromycin B and pluramycin. Lanes 1, 2, 3, and 4 contain 50, 100, 150, and 300 ng of drug molecules, respectively. For both (A) and (B), MS and DS refer to monomeric and dimeric supercoiled forms of DNA. MR and DR are relaxed forms of DNA from the supercoiled form of the same species of DNA.

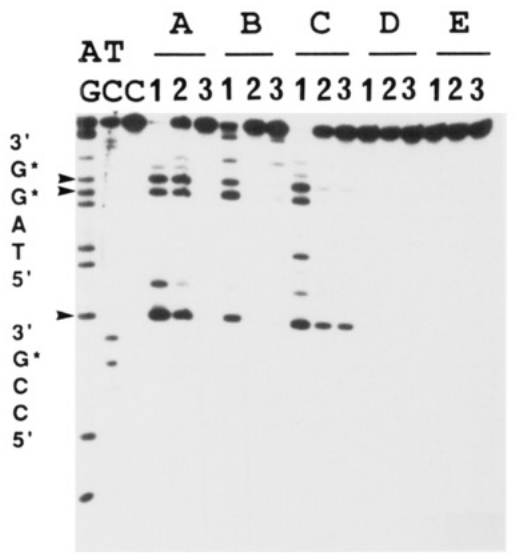

FIGURE 3: Comparison of the alkylating ability of the pluramycin antibiotics using the oligomer duplex. Panels A–E contain altromycin H, altromycin I, hedamycin, neopluramycin, and kidamycin, respectively. AG and TC refer to the purine- and pyrimidine-specific chemical cleavage reaction. Lane C is without drug treatment, and lanes 1–3 contain 60, 20, and 5 ng of drug molecules, respectively. The reaction of DNA (20 ng) with drug molecules was carried out for 12 h at room temperature. The sequences alkylated are shown to the left of the gel, the asterisk indicates covalently modified guanines, and the arrowheads correspond to these guanines.

species of sc DNA appear, which are a monomer (MS) and dimer (DS) pair. The conversion of sc (MS and DS, form I) to relaxed circular (MR and DR, relaxed form I) by topo I is evident from the comparative migration of the bands in lanes SC and R in Figure 2. For altromycin B and hedamycin, both relaxed species migrate slightly slower with increasing amount of drug, presumably due to covalent modification to DNA, resulting in the structural changes in DNA molecules. No direct evidence of intercalation is visible in this assay, which includes topo I. In contrast, for kidamycin and neopluramycin, well-defined bands representing different topological forms, which migrated faster than the relaxed form I, were present after incubation at the higher drug levels (lanes 3 and 4). In a second experiment using sc DNA modified with altromycin B and pluramycin, but *without* topo I (Figure 2B), the highest concentration of drug produced a smear of bands for MS and DS running slower than sc DNA, indicative of intercalation accompanying covalent adduct formation (lane 4). This is exactly as expected, since intercalation would retard migration, and in the absence of topo I to relax the unwinding by altromycin B and pluramycin, this would appear as a smear of bands. Therefore, this result, taken in conjunction with the results of the sc DNA assay, including topo I with the noncovalently modifying pluramycins (kidamycin and neopluramycin), provides excellent evidence for an intercalative type of binding to DNA common to all members of the pluramycin family with or without associated alkylation.

While Altromycins H and I and Hedamycin Covalently Modify Guanine in DNA, Neopluramycin and Kidamycin Are Devoid of DNA Alkylation Ability. To follow up the results of the agarose gel electrophoresis experiments (Figure 2), an experiment using high-resolution gel electrophoresis was carried out to test our hypothesis that while the altromycins and hedamycin both intercalate *and* covalently modify DNA, kidamycin and pluramycin are only able to intercalate into DNA. An oligomer (I in Table I) was singly labeled with ^{32}P at the 5′ end of the (+) strand and incubated with each of these drugs in separate experiments. Each sample was subjected to thermal treatment (95 °C for 30 min) in the presence of 1 M piperidine prior to loading on the gel. The results show that strand breakage at several guanine residues (arrows in Figure 3) was found for both altromycins H and I and hedamycin (A, B, and C in Figure 3). In contrast, neopluramycin and kidamycin (D and E in Figure 3) did not produce DNA strand breakage. The results of this experiment support our proposal that the epoxide common to both altromycins H and I and hedamycin is required for alkylation of DNA.

Altromycin B Alkylation at Guanine Occurs at N3 or N7. Several DNA-damaging drugs produce strand breakage as a consequence of heat or alkali treatment of alkylated DNA. Probable alkylation positions on guanine include 2N, N3, or N7. The exocyclic 2-amino group (2N) can be eliminated since this would not result in strand breakage of DNA after heat or alkali treatment. Both N3 and N7 alkylation would be expected to produce strand breakage, and of these N7 seems the most likely on the basis of the well-established occurrence of N7-alkylated guanines (Warpehoski & Hurley, 1988). In either case (N3 or N7 alkylation) depurination leads to an AP site, which can then undergo two successive β-eliminations. For 3′-labeled DNA, the first β-elimination leads directly to a product that comigrates with a Maxam and Gilbert guanine reaction, but for 5′-labeled DNA, two successive β-eliminations are required, the second of which requires alkali treatment for completion. The results in Figure 4 show the expected pattern of cleavage at guanine consistent with either N3 or N7 alkylation; i.e., 3′ ^{32}P-labeled oligomer II (Table I) shows a similar pattern with either heat or heat

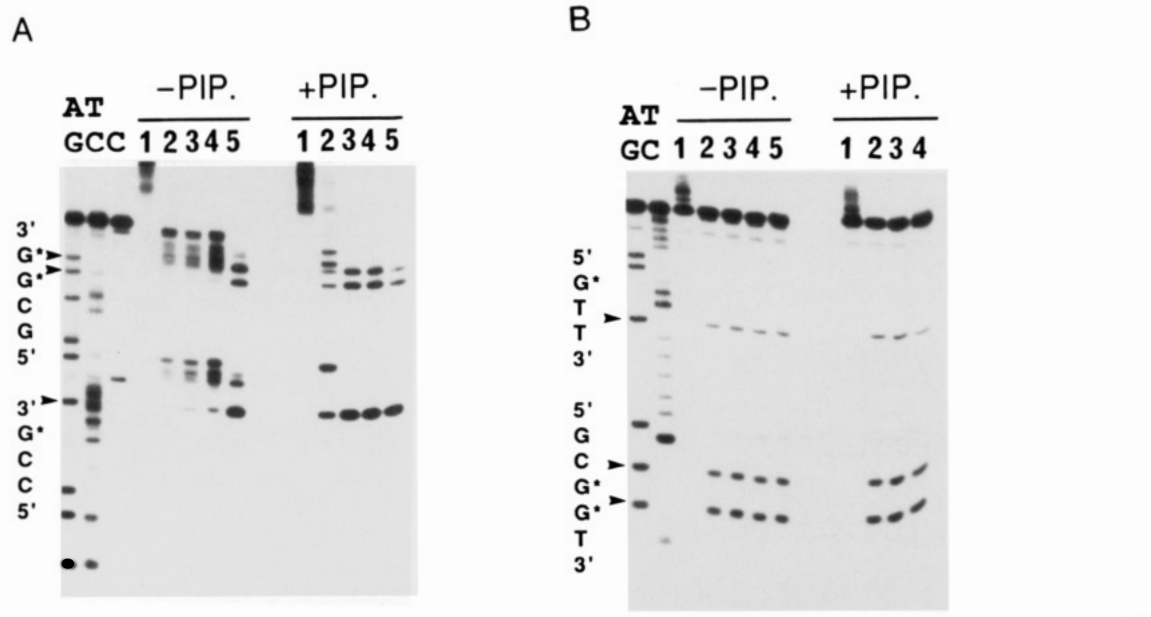

FIGURE 4: Thermal treatment of altromycin B-modified 3′ and 5′ end-labeled DNA with (+PIP) or without (−PIP) piperidine. In panel A, 5′ end-labeled oligomer DNA B (lower strand) and, in panel B, 3′ end-labeled oligomer DNA B (lower strand) were used in these experiments. DNA (10 ng in panel A and 30 ng in panel B) was treated with 60 ng of altromycin B for 12 h, and DNA was purified with phenol/chloroform extraction and ethanol precipitation to remove unbound drug molecules. In each case, lanes 1–5 contain DNA samples heated at 95 °C for 0, 4, 10, 20, and 30 min, respectively. For sequences to the left of the gel, see the legend for Figure 3.

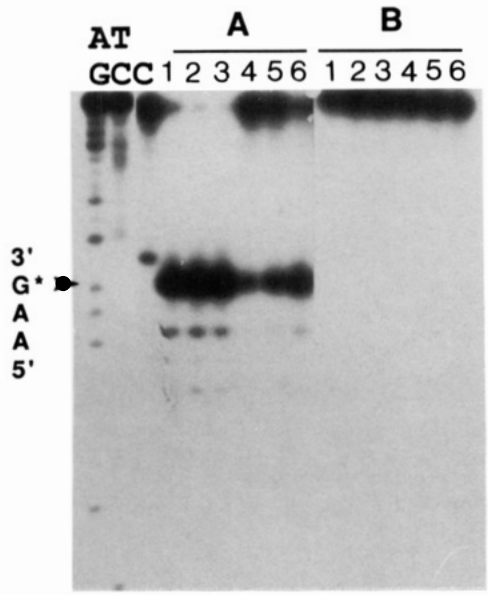

FIGURE 5: Reactivity of altromycin B and pluramycin with oligomer C containing either guanine or N^7-deazaguanine at the * site (see Table I). Panel A refers to oligomer C, and panel B refers to the same oligomer but containing N^7-deazaguanine on the (+) strand at the * site instead of guanine. Oligomer C was labeled at the 5′ end of the (+) strand. Lane C is control experiment without drug treatment. Lanes 1–3 (and 4–6) contain 10 ng, 50 ng, and 150 ng of altromycin B (or pluramycin), respectively, each with 20 ng of oligomer.

and piperidine treatment, but the 5′ ^{32}P-labeled oligomer required heat *and* piperidine for completion of the chemical degradation reaction leading to a product that comigrates with a Maxam and Gilbert sequencing reaction for guanine.

N^7-Deazaguanine Is Not a Substrate for Alkylation by Altromycin B or Pluramycin. To potentially distinguish between N3 and N7 alkylation of DNA by altromycin B, duplex oligomers (III in Table I) containing either a single guanine or N^7-deazaguanine for alkylation on the upper strand were prepared. Both duplexes containing either guanine or N^7-deazaguanine at this unique position on the upper strand were reacted with altromycin B and pluramycin, and alkylation was monitored by the strand breakage assay (see before). While altromycin B and pluramycin produced strand breakage at the guanine residue (lanes 1–6 in panel A of Figure 5), the oligomer containing the N^7-deazaguanine was unreactive, potentially implicating N7 as the alkylation site for both altromycin B and pluramycin.[2]

Characterization of the Altromycin B–Guanine Adduct by Mass Spectrometry and NMR. Because of the uncertainty of the results described above, further characterization of the site for alkylation of DNA was important. The altromycin B–guanine adduct was isolated from a sample of calf thymus DNA that had been incubated with altromycin B and subsequently heated at 90 °C to produce depurination and release of the guanine adduct. The butanol extract containing the altromycin B–guanine adduct was lyophilized and subjected to mass spectrometric and NMR analysis.

Positive ion mode FAB-MS of the altromycin B–base adduct gave a parent ion (M + 1) of 1077.429242 (calculated 1077.430450), which is equivalent to a molecular formula of $C_{52}H_{65}N_6O_{19}$. This is consistent with the structure of the altromycin B–guanine adduct shown in Figure 6A. ^{13}C- and ^{1}H-NMR spectra were obtained on a further sample of the same material. A comparison of the chemical shifts of the proton NMR resonance signals of altromycin B, the altromycin B–guanine adduct, and N^7-methylguanine is shown in Table II. Characteristic of a 7-alkylated guanine adduct is the presence of just one nonexchangeable proton resonance signal (H8) and the two exchangeable resonance signals for $2NH_2$ and N1-*H*. The proton NMR chemical shift of H16 of altromycin is consistent with nucleophilic attack at C16 and acid-catalyzed opening of the epoxide ring. The ^{13}C-NMR chemical shifts of C14 and C16 (76 and 59 ppm) in the altromycin B–guanine adduct in comparison to altromycin B (59.7 and 67.5 ppm) are also consistent with attachment of nitrogen (N7 of G) at C16. Further evidence for the covalent linkage sites was obtained from ^{1}H–^{13}C and ^{1}H–^{15}N HMBC experiments (Bax & Summers, 1986). The long-range ^{1}H–^{13}C coupling indicates an intact carbon backbone extending

[2] In control experiments with N3 alkylation of N^7-deazaguanine, an adduct is produced, providing assurance that an adduct would form at N3 with altromycin B if this was the alkylation site.

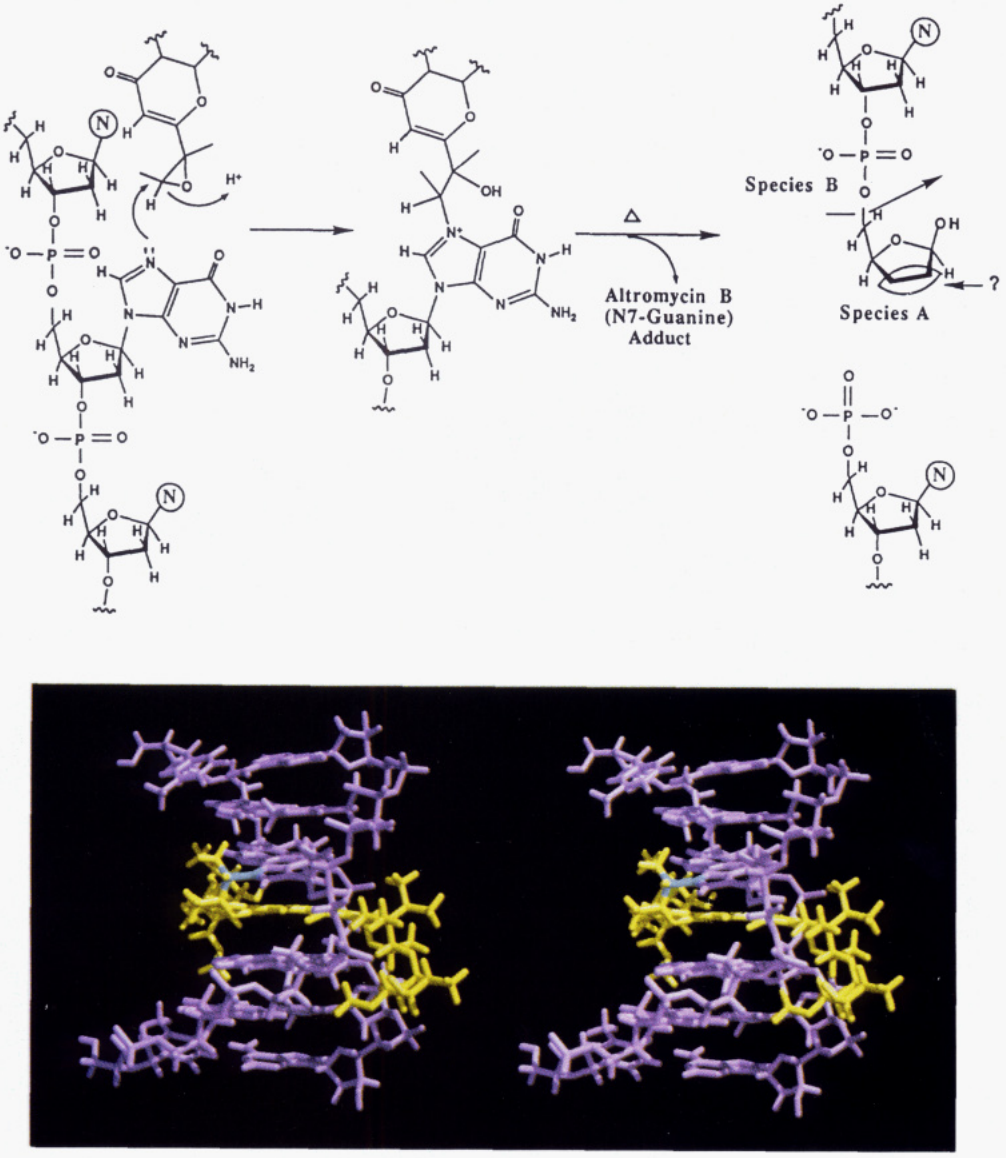

FIGURE 6: (A, top) Proposed reaction of altromycin B with DNA to form the altromycin B (N7-guanine)–DNA adduct and products of thermal and piperidine cleavage of the DNA adduct. Species A is the product of thermal cleavage at neutral pH, and species B is the product after alkaline treatment. (B, bottom) Stereodiagram of the altromycin B–DNA adduct prepared using AMBER 4.0. The starting structure was derived by modifying DNA coordinates from the daunomycin–DNA complex (Quigley et al., 1980) and docking altromycin B to this oligomer. The covalent linkage site is shown in blue and the altromycin B molecule in yellow.

from C14 to C16, and the ^{1}H–^{15}N experiment shows long-range coupling between the 17-methyl of altromycin and N7 of guanine (supplementary material). Last, through-space connectivities in the ROESY experiment between H8 of guanine and the 17-methyl are also consistent with N7 rather than N3 alkylation of guanine (unpublished results). In summary, the ^{1}H- and ^{13}C-NMR confirms N7 as the alkylation site on guanine and demonstrates that the epoxide is the reactive species that is opened up by nucleophilic attack at C16 to give the alkylation product shown in Figure 6.

DISCUSSION

The altromycins (Figure 1) are members of the pluramycin group of antibiotics. Like most other members of the pluramycin family of antibiotics, the altromycins have potent antitumor activity that is believed to be related to their ability to interact with DNA. Although the altromycins appear to interact in both grooves of DNA (McAlpine et al., 1992), a general understanding of their interaction with DNA has remained elusive. In this study we have demonstrated that altromycin B and the related pluramycins intercalate between base pairs and, where an epoxide is present, alkylate N7 of guanine. On the basis of these data and a knowledge of the threading mechanism for the interaction of the anthracycline nogalamycin with DNA (see later), a structure of the altromycin B (N7-guanine)–DNA adduct is proposed (Figure 6B).

From a strictly structural perspective, the altromycin B molecule can be divided into four distinct regions: the planar aglycon moiety, which consists of a 4*H*-anthra[1,2-*b*]pyran ring system, the disaccharide at C10, the glycosidically bound neutral sugar at C5, and the epoxide at C2 (Figure 1). A major lead in predicting how the altromycins might interact with DNA is the recognition that this aglycon moiety is similar in structure to nogalamycin and the synthetic anthracene-9,10-dione group of antitumor antibiotics (Tanious et al., 1992), which interact with DNA through a "threading mechanism" (Islam et al., 1985). A key structural feature of DNA-threading compounds is the presence of bulky side chains or rings at two or more corners of a planar anthraquinone ring system. This is clearly the case for the altromycins (substituents at C2, C5, and C10) and also for other members of the pluramycin group (e.g., substituents at C2, C8, and C10).

The prototypic DNA-threading compound is the anthracycline nogalamycin. The threading mode of interaction of

Table II: ^{1}H-NMR Chemical Shifts of Altromycin B, Altromycin B–Guanine (AltB–G) Adduct, and N^7-Methylguanine

	chemical shifts (ppm)			chemical shifts (ppm)	
^{1}H assignment[a]	altromycin B[b]	AltB–G adduct[c]	^{1}H assignment[a]	altromycin B[b]	AltB–G adduct[c]
3	6.52	6.30	5a″	2.48	2.40
6	8.69	8.65	5b″	1.25	1.25
8	7.85	7.85	6″	5.53	5.50
9	7.98	7.95	2″Me	1.65	1.68
15	1.93	1.88	4″Me	1.04	1.05
16	3.35	5.55	4″N(Me)$_2$	2.47	2.45
17	1.25	1.65	1‴	4.79	4.75
2′	4.31	4.40	2a‴	2.31	2.15
3′	3.96	3.72	2b‴	1.78	1.75
4′	3.59	3.59[d]	3‴	3.62	3.55
5′	4.66	4.67[d]	4‴	3.27	3.25
6′	4.56	4.47[d]	5‴	4.09	4.05
2′Me	1.73	1.50	5‴Me	1.26	1.15
2″	4.32	4.35	Gua 8		7.95 (7.90)[e]
3″	3.77	3.65	Gua N2H$_2$		5.85 (6.00)
Gua N1H		10.35 (10.75)			

[a] For numbering, see Figure 1. [b] Chemical shifts taken from Brill et al. (1990). [c] Chemical shifts measured at 500 MHz in 4:1 deuterated DMSO/benzene at 333 K. [d] Tentative assignments. [e] Numbers in parentheses are chemical shifts of N^7-methylguanine measured at 500 MHz in deuterated DMSO at 300 K.

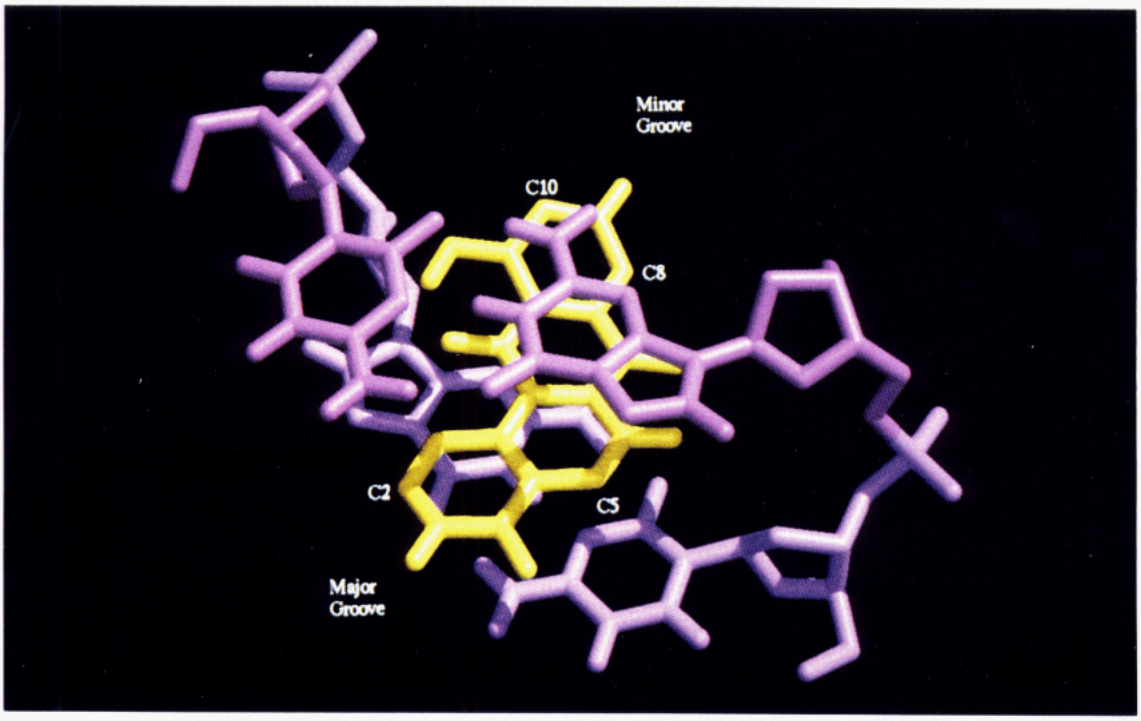

FIGURE 7: General threaded/intercalated model of the pluramycin (N7-guanine)–DNA adduct showing the positioning of substituents C2 and C5 in the major groove and C8 and C10 in the minor groove.

nogalamycin with DNA has been thoroughly characterized using kinetics (Gabbay et al., 1973; Fox & Waring, 1984; Fox et al., 1985; Tanious et al., 1991), molecular modeling (Collier et al., 1984), X-ray crystallographic (Arora, 1983; Wang et al., 1990; Liaw et al., 1989; Williams et al., 1990), and NMR data (Searle et al., 1988; Zhang & Patel, 1990; Van Houte et al., 1992). This threading type of DNA binding is differentiated from classical intercalation because, in the case of threading, *two* side chains or rings lie one in each of the major and minor DNA grooves, rather than just the *one* groove occupied for classic intercalators (Baguley, 1991).

On the basis of the known structure of the nogalamycin–DNA complex and the knowledge that altromycin B alkylates N7 of guanine in the major groove, a structure of the threaded altromycin B (N7-guanine)–DNA adduct is proposed (Figure 6B). In this model the 4*H*-anthra[1,2-*b*]pyran ring intercalates between the base pairs and positions the side chains at C2 (epoxide) and C5 (*C*-glycoside) in the major groove and the disaccharide at C10 in the minor groove of DNA. There is necessarily a disruption of DNA structure in order to accommodate threading of a bulky side chain from one side to another. This process could be most easily accommodated if the threading takes place *from* the major *to* the minor groove. The intercalative binding of the 4*H*-anthra[1,2-*b*]pyran ring then positions the epoxide in the major groove in proximity to N7 of guanine to facilitate nucleophilic attack at C16 and acid-catalyzed opening of the epoxide ring (Figure 6A). Thus, the final drug–DNA adduct is an intercalated threaded complex that is covalently bound through N7 of guanine in the major groove of DNA.

In the proposed model the intercalation site is placed arbitrarily between the covalently modified GC base pair and the base pair to the 5′ side of the covalently modified guanine. Although the sequence specificity lies to the 5′ side of the covalently modified guanine (5′ AG*N), it is most likely that this preference is due to groove binding interactions rather

than the intercalative binding interactions, since different members of the pluramycin family (altromycin *vs* hedamycin) have different sequence specificities (Sun and Hurley, unpublished results), although they possess the same intercalative moiety.

This intercalative threading interaction with associated alkylation for altromycin B can be extended to propose a general model (Figure 7) for the interaction of the pluramycins with DNA. In this model the 4*H*-anthra[1,2-*b*]pyran ring intercalates between the base pairs as shown, and the side chains at C2 and C5 are in the major groove and those at C8 and C10 are in the minor groove. Presumably, these side chains interact either noncovalently (e.g., sugars) or covalently (e.g., epoxide). This model provides a novel motif for a conjoint intercalation–alkylation interaction with DNA that also incorporates a threading mechanism. Studies are in progress using high-field NMR on a defined oligomer to provide more precise data on the proposed altromycin B–DNA adduct structure.

Perhaps the most definitive data previously obtained on the interaction of other members of the pluramycin group of antitumor antibiotics with DNA is available from studies on hedamycin (White & White, 1969; Jernigan et al., 1978; Bennett, 1982). In the earlier work (White & White, 1969; Jernigan et al., 1978), two types of DNA binding by hedamycin were determined, corresponding to irreversible (type I) and reversible (type II). In both cases, intercalation is proposed to be involved with, in addition, covalent bonding for type I binding. In the more recent work, Bennett (1982) has shown that hedamycin forms a stable complex with DNA with associated alkali-labile strand breaks that occur at deoxyguanosine. He also reported that 5′ TG sequences are especially reactive to hedamycin. On the basis of analogies with other alkylating species, such as dimethyl sulfate, N7 of guanine was postulated to be the reactive site. These data are in accord with our proposed general model for the reaction of the pluramycin group of antibiotics with DNA.

Finally, it seems likely that the mechanism of action of kapuramycin A3, a tetrahydroanthrapyrone antitumor antibiotic produced by a *Streptomyces* species (Hara et al., 1990), and aflatoxin B_1 is related to that of the pluramycins. Kapuramycin A3 has been shown to produce covalent modification of guanine either through N3 or N7 in DNA and also produce single-strand scission of DNA. On the basis of the result described here for altromycin B, we propose that N7 of guanine is the alkylation site, and the β,γ-unsaturated δ-keto carboxylic acid threads into the minor groove and catalyzes the strand breakage of DNA by an as yet undetermined mechanism. Aflatoxin B_1 is quite analogous to the covalently modifying pluramycins in that it intercalates into DNA and alkylates N7 of guanine via an epoxide (Gopalakrishnam et al., 1990). However, from a structural perspective, the pluramycins and aflatoxin B_1 are quite different.

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SUPPLEMENTARY MATERIAL AVAILABLE

Long-range ^1H–^{13}C (S1) and ^1H–^{15}N (S2) experiments showing the coupling between C14 and C16 (S1) and the coupling between the 17-Me of altromycin B and N7 of guanine (S2) (2 pages). Ordering information is given on any current masthead page.

REFERENCES

Arora, S. K. (1983) *J. Am. Chem. Soc. 105*, 1328–1332.
Baguley, B. (1991) *Anti-Cancer Drug Des. 6*, 1–35.
Bax, A., & Summers, M. F. (1986) *J. Am. Chem. Soc. 108*, 2093–2094.
Bennett, G. N. (1982) *Nucleic Acids Res. 10*, 4581–4594.
Brill, G. M., McAlpine, J. B., Whittern, D. N., & Buko, A. M. (1990) *J. Antibiot. 43*, 229–237.
Byrne, K., Gonda, S. K., & Hilton, B. D. (1985) *J. Antibiot. 38*, 1040–1049.
Collier, D. A., Neidle, S., & Brown, J. R. (1984) *Biochem. Pharmacol. 33*, 2877–2880.
Fox, K. R., & Waring, M. J. (1984) *Biochim. Biophys. Acta 802*, 162–168.
Fox, K. R., Brassett, C., & Waring, M. J. (1985) *Biochim. Biophys. Acta 840*, 383–392.
Gabbay, E. J., DeStefano, R., & Baxter, C. S. (1973) *Biochem. Biophys. Res. Commun. 51*, 1083–1089.
Gopalakrishnam, S., Harris, T. M., & Stone, M. P. (1990) *Biochemistry 29*, 10438–10442.
Hara, M., Yoshida, M., & Hakano, H. (1990) *Biochemistry 29*, 10449–10455.
Islam, S. A., Neidle, S., Gandecha, B. M., Partridge, M., Patterson, L. H., & Brown, J. R. (1985) *J. Med. Chem. 28*, 857–864.
Jackson, M., Karwowski, J. P., Theriault, R. J., Hardy, D. J., Swanson, S. J., Barlow, G. J., Tillis, P. M., & McAlpine, J. B. (1990) *J. Antibiot. 43*, 223–228.
Jernigan, H. M., Jr., Irvin, J. L., & White, J. R. (1978) *Biochemistry 17*, 4232–4238.
Lee, C.-S., Sun, D., Kizu, R., & Hurley, L. H. (1991) *Chem. Res. Toxicol. 4*, 203–213.
Liaw, Y.-C., Gao, Y.-G., Robinson, H., van der Marel, G. A., van Boom, J. H., & Wang, A. H.-J. (1989) *Biochemistry 28*, 9913–9918.
Maxam, A. M., & Gilbert, W. (1980) *Methods Enzymol. 65*, 499–560.
McAlpine, J. B., Karwowski, J. P., Jackson, M., Brill, G. M., Kadam, S., Shen, L., Clement, J. J., Alder, J., & Burres, N. S. (1993) in *Antitumor Drug Discovery and Development* (Valeriote, F. A., Corbett, T. H., & Baker, L. H., Eds.) Kluwer Academic Publishers, Boston, MA (in press).
Quigley, G. J., Wang, A. H.-J., Ughetto, G., van der Marel, G., van Boom, J. H., & Rich, A. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 7204–7208.
Searle, M. S., Hall, J. G., Denny, W. A., & Wakelin, L. P. G. (1988) *Biochemistry 27*, 4340–4349.
Sequin, U. (1986) *Fortschr. Chem. Org. Naturst. 50*, 57–122.
Tanious, F. A., Yen, S. F., & Wilson, W. D. (1991) *Biochemistry 30*, 1813–1819.
Van Houte, L. P. A., van Garderen, C. J., & Patel, D. J. (1983) *Biochemistry 32*, 1667–1674.
Wang, A. H.-J., Liaw, Y.-C., Robinson, H., & Gao, Y.-G. (1990) in *Molecular Basis of Specificity in Nucleic Acid–Drug Interactions* (Pullman, B., & Jortner, J., Eds.) Jerusalem Symposia on Quantum Chemistry and Biochemistry, Vol. 23, pp 1–21, Kluwer Academic Publishers, Dordrecht, The Netherlands.
Warpehoski, M. A., & Hurley, L. H. (1988) *Chem. Res. Toxicol. 1*, 315–333.
White, H. L., & White, J. R. (1969) *Biochemistry 8*, 1030–1042.
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. U.S.A. 87*, 2225–2229.
Yasuzawa, T., Saitoh, Y., & Sano, H. (1990) *J. Antibiot. 43*, 485–491.
Zhang, X., & Patel, D. J. (1990) *Biochemistry 29*, 9451–9466.