Naphthyridinomycin, a DNA-Reactive Antibiotic

MILTON J. ZMIJEWSKI, JR.,* KRYN MILLER-HATCH, AND MARCIA GOEBEL

College of Pharmacy, Department of Medicinal Chemistry, University of Utah, Salt Lake City, Utah 84112

Received 2 November 1981/Accepted 1 February 1982

Naphthyridinomycin is a novel quinone antibiotic that is produced in liquid shake cultures by *Streptomyces lusitanus*. Fermentation studies have shown that this antibiotic is produced maximally after 96 h of cell growth. L-[methyl-³H]methionine efficiently labels naphthyridinomycin when it is added to a fermentation mixture 24 h before culture is harvested. Unlabeled and radioactively labeled naphthyridinomycin were used to determine the mechanism of action of this unique antibiotic. Naphthyridinomycin inhibited bacterial growth primarily by inhibiting DNA synthesis. The structural similarity between naphthyridinomycin and the saframycins suggested that naphthyridinomycin might inhibit DNA synthesis by binding to the template. In vitro studies with radiolabeled naphthyridinomycin indicated that this antibiotic does specifically bind to calf thymus DNA. The binding reaction was enhanced by adding sulfhydryl-containing compounds; dithiothreitol was the best activating agent. DNA-naphthyridinomycin complexes were a poor substrate for enzymes that catalyze DNA-directed DNA and RNA syntheses. These results showed that naphthyridinomycin is similar to the saframycins in its reactivity toward DNA and suggested that the mechanism by which naphthyridinomycin inhibits DNA synthesis is through its ability to bind specifically to the DNA template of the cell.

Naphthyridinomycin was isolated from *Streptomyces lusitanus*, a novel streptomycete found in an Easter Island soil sample (10). The structure and stereochemistry of this compound have been determined by Sygusch et al. (13) (Fig. 1). Naphthyridinomycin was found to possess potent antimicrobial activity against gram-positive and gram-negative bacteria. In a previous study (12), Singh et al. found that at low concentrations this antibiotic specifically inhibits bacterial DNA synthesis and that at higher concentrations it also prevents RNA synthesis and protein synthesis. Other cell functions, such as respiration, cell wall synthesis, and cell membrane integrity, are not drastically affected by this antibiotic (12). The molecular mechanism by which naphthyridinomycin inhibits DNA synthesis was not determined by Singh et al.

Naphthyridinomycin is structurally related to the saframycins (1-3), in particular saframycin S (3). Saframycins A bind to DNA after activation by sulfhydryl-containing compounds (9). The saframycins also have a more pronounced effect on bacterial DNA synthesis than on RNA synthesis (8). The results of studies with the saframycins and the structural similarity of these antibiotics to naphthyridinomycin suggested that naphthyridinomycin might also bind to DNA and, in doing so, inhibit template-directed DNA and RNA syntheses.

Biosynthetic studies on naphthyridinomycin

(Zmijewski, unpublished data) made available radioisotope-labeled naphthyridinomycin for mechanism-of-action studies. The results presented here suggest that this antibiotic mimics saframycin S in its reactivity toward DNA. Since reference naphthyridinomycin was not available, we also describe the fermentation used to prepare radiolabeled antibiotic, as well as spectral data which confirm that we were working with the antibiotic isolated previously (10).

MATERIALS AND METHODS

Production of naphthyridinomycin. *S. lusitanus* NRRL 8054 was used as the source of naphthyridinomycin (9). This organism was maintained on sterile soil. For antibiotic production, a seed medium (100 ml in a 500-ml Erlenmeyer flask) containing 1.0% malt extract, 0.4% yeast extract, and 0.4% glucose (pH 7.3) was inoculated with a loopful of the soil culture. The organism was grown at 25°C with shaking (250 rpm) for 48 h; 5 ml of this seed culture was used to inoculate 100 ml of antibiotic production medium containing 2.0% glucose, 0.8% peptone, 1.0% tomato paste, 0.8% corn meal, 2.0% molasses, and 0.3% sodium chloride. The pH of the medium was adjusted so that the pH after autoclaving was 7.4. The pH of the fermentation broth was maintained near 6.0 during cell growth by periodic additions of 0.2 mM imidazole buffer (pH 7.8). Antibiotic synthesis reached a maximum after 96 h of growth.

Purification of naphthyridinomycin. Production cultures of *S. lusitanus* were harvested after 96 h of cell

growth. The pH of the broth was adjusted to 4.0, and the mycelium was filtered off. The fermentation beer was then adjusted to pH 10.0 and extracted with methylene chloride. After evaporation of the methylene chloride, the residue was applied to an alumina column (activity V; J. T. Baker Chemical Co.), and the antibiotic was eluted with benzene-chloroform (80:20). Naphthyridinomycin has a bright orange-red color, and the colored fractions from the column were pooled. This material was then applied to a second alumina column (activity V), and the antibiotic was eluted with benzene-methanol-acetone (90:5:5). In the last step of the purification, the antibiotic was applied to reverse-phase thin-layer chromatography plates (ODS on silica; Whatman), and the plates were developed in methanol-water (80:20). The antibiotic was eluted from the developed chromatogram with chloroform. The yield of purified antibiotic ($\epsilon_{270\text{nm}}$, 10,354) was between 1 and 2 mg/100 ml of culture broth. The UV spectra of the compound were recorded with a Beckman model DU-8 spectrophotometer, the infrared spectra were recorded with a Beckman Aculab 1 infrared spectrometer, and the nuclear magnetic resonance spectra were recorded with a JEOL model FX-270 multinuclear spectrometer.

Preparation of [^3H]naphthyridinomycin. In a typical labeling experiment, 50 μCi of L-[methyl- ^3H]methionine (80 Ci/mmol; New England Nuclear Corp.) was added to 100 ml of a production culture of *S. lusitanus* at the appropriate time. The labeled antibiotic was extracted and purified as described above.

Test bacteria and growth conditions. All experiments requiring test organisms were performed with *Escherichia coli* LC462, which required thiamine, thymine, and serine (kindly supplied by Dana Carroll, Department of Cellular, Viral and Molecular Biology, University of Utah). Bacteria were grown in either brain heart infusion broth or medium H1, which contained 1.05 mg of L-serine, 560 mg of K_2HPO_4 , 240 mg of KH_2PO_4 , 100 mg of $(\text{NH}_4)_2\text{SO}_4$, 0.1 ml of 1 mM FeCl_3 , 1 ml of 5% Casamino Acids, 100 μg of thymine, 0.08 ml of 1 M MgSO_4 , 0.19 ml of 20% glucose, and 0.04 ml of a thiamine solution (10 mg/ml) in a final volume of 50 ml. Bacterial growth was monitored by measuring the absorbance of cultures at 660 nm. Viable cell counts were determined by plating serial dilutions (in distilled water) of a culture onto brain heart infusion agar. In a typical experiment, *E. coli* LC462 was grown overnight in the test medium, and 1 ml of the overnight broth was inoculated into 50 ml of fresh medium and incubated at 37°C with shaking (100 rpm) for either 1 h (brain heart infusion broth) or 3.5 h (medium H1). A 5-ml portion of this broth was used to inoculate 50 ml of fresh medium, and the resulting preparation was incubated in the same fashion.

Measurement of cellular macromolecular synthesis. DNA synthesis, RNA synthesis, and protein synthesis were monitored by determining the incorporation of [methyl- ^3H]thymine (58.7 Ci/mmol; New England Nuclear Corp.), [2- ^{14}C]uracil (55.2 mCi/mmol; New England Nuclear Corp.), and L-[U- ^{14}C]leucine (341 mCi/mmol; New England Nuclear Corp.), respectively, into a cold trichloroacetic acid (TCA) precipitate. Flasks containing 9 ml of medium H1 were inoculated with 1 ml of a 3.5-h culture of *E. coli* LC462. Labeled precursors were added (final concentration, 0.1 μCi /ml) along with the appropriate concentration of

FIG. 1. Structure of naphthyridinomycin.

naphthyridinomycin. The cultures were incubated at 37°C with shaking (100 rpm). After 90 min, 1.0-ml samples were added to 2 ml of 10% TCA and placed on ice for 30 min. The acid-insoluble material was collected on membrane filters (pore size, 0.45 μm ; Millipore Corp.). The filters were washed with ice-cold TCA and ethanol and then air dried. The filters containing the TCA precipitates were placed in scintillation vials containing 1 ml of 0.5 N HCl, and the sealed vials were heated at 90°C for 45 min. RNA-containing samples were treated similarly, except that these samples were heated with 0.5 N ammonium hydroxide. Ethyl acetate (1.0-ml portions) was added to the cooled vials to dissolve the membrane filters. After dissolution of the filters, 10-ml portions of Aquasol (New England Nuclear Corp.) were added, and the samples were counted for radioactivity with a Beckman model LS-9000 or model LS-7800 liquid scintillation counter.

Binding of [^3H]naphthyridinomycin to DNAs. Dialysis experiments were used to determine the extent of binding of [^3H]naphthyridinomycin to DNAs and other cellular macromolecules. [^3H]naphthyridinomycin (0.057 μmol /ml), DNA (0.42 μmol of nucleotide base pairs per ml, in most cases), and a reducing reagent (the concentration depended on the experiment) were incubated in 0.05 M Tris-hydrochloride buffer (pH 7.9) at room temperature. After 2 h, the reaction mixture (usually 5 ml) was placed in a dialysis bag and dialyzed at 4°C for 48 h against two changes (250 ml each) of the same buffer. After 2 days, the solutions in the dialysis bag were counted for radioactivity.

Calf thymus DNA, yeast bulk RNA, polydeoxyguanydic acid-polydeoxycytidylic acid, and polydeoxyadenylic acid-polydeoxythymidylic acid were purchased from Sigma Chemical Co. Nucleic acid concentrations were calculated by making the following assumptions: $\epsilon_{260\text{nm}} = 6,700$ (calf thymus DNA); $\epsilon_{259\text{nm}} = 6,000$ (polydeoxyadenylic acid-polydeoxythymidylic acid); $\epsilon_{253\text{nm}} = 7,400$ (polydeoxyguanydic acid-polydeoxycytidylic acid); and $\epsilon_{260\text{nm}} = 7,700$ (yeast RNA).

DNA and RNA polymerases. *E. coli* DNA-dependent DNA polymerase (Sigma Chemical Co.) was assayed as described by Lehman (11). *E. coli* DNA-dependent RNA polymerase (Sigma Chemical Co.) was assayed by the method of Burgess (4). In both cases, β -mercaptoethanol was omitted from the assay mixture. This did not appreciably affect enzyme activity, perhaps because a specified amount of a sulfhydryl com-

FIG. 2. Time course for growth (○), antibiotic production (△) and incorporation of L(methyl-³H)methionine (□) into naphthyridinomycin by *S. lusitanus* in 100 ml of production medium.

pound was already present in the freeze-dried enzyme preparations. DNA synthesis was monitored by following the incorporation of [methyl-³H]dTTP (sodium salt; 20.2 Ci/mol; New England Nuclear Corp.) into a TCA precipitate. Likewise, [8-³H]GTP (sodium salt; 7.5 Ci/mol; New England Nuclear Corp.) was used to follow RNA synthesis. The total volume of each reaction mixture was increased to 250 µl for ease of handling.

RESULTS

Production of naphthyridinomycin by *S. lusitanus*. The growth of *S. lusitanus* and the time course for naphthyridinomycin production are shown in Fig. 2. Very little antibiotic was synthesized before 72 h of cell growth. Maximum antibiotic synthesis occurred at 96 h, and thereafter the antibiotic that could be isolated from the fermentation broth decreased rapidly. The time course for the fermentation was consistent with previously reported batch fermentations of *S. lusitanus* (10).

Since reference antibiotic was not available, it was necessary to establish that the antibiotic being produced by *S. lusitanus* was, in fact, naphthyridinomycin. The antibiotic was isolated from the fermentation broth and exhibited the ruby red color of naphthyridinomycin, as well as the correct UV absorption maximum at 270 nm (ϵ_{max} , 10,354). The infrared spectrum of the antibiotic showed characteristic absorption bands at 3,000, 2,940, 2,845, 1,715, 1,690, 1,607, and 1,495 cm^{-1} . The proton nuclear magnetic resonance of the antibiotic was characterized by three singlets at 4.1, 2.40, and 1.95 ppm from tetramethylsilane due to the protons of the three methyl groups of naphthyridinomycin. All three of the spectra described above agreed exactly with the previously reported spectra of naphthyridinomycin (10).

Since radiolabeled naphthyridinomycin would be a decisive advantage in our mechanism-of-action studies, we used a biosynthetic method to

TABLE 1. Successive purification steps and specific activities of [³H]naphthyridinomycin

Purification step	Conditions	Sp act (µCi/µmol)
1	Alumina (activity V) eluted with benzene-chloroform (80:20)	0.61
2	Alumina (activity V) eluted with benzene-methanol-acetone (95:5:5)	0.66
3	ODS reverse-phase thin-layer chromatography plates developed with methanol-water (80:20) and eluted with chloroform	0.61

prepare [³H]naphthyridinomycin. The structure of naphthyridinomycin suggested that at least two of the methyl groups (O and N methyls) should be derived from the S-methyl group of methionine. The addition of L-[methyl-³H]methionine to production cultures of *S. lusitanus* resulted in antibiotic that was labeled to the greatest extent during maximum antibiotic synthesis (Fig. 2). The maximum incorporation (2% of the label added) of the precursor occurred when it was added after 72 h of cell growth and the antibiotic was isolated 24 h later. Successive purifications of the antibiotic resulted in no significant change in the specific activity of the tritium-labeled antibiotic (Table 1), indicating that the label was associated with the antibiotic.

Inhibition of growth and macromolecular synthesis of *E. coli* by naphthyridinomycin. After we established that the antibiotic produced by *S. lusitanus* was indeed naphthyridinomycin, it seemed reasonable to also demonstrate that the antibiotic was effective in inhibiting the growth of *E. coli* at the same concentration as previously reported (12). As monitored by changes in

TABLE 2. Effect of naphthyridinomycin on DNA synthesis, RNA synthesis, and protein synthesis in *E. coli* LC462 grown on medium H1

Concn (µg/ml)	Inhibition of incorporation of label into macromolecules (%) ^a		
	[methyl- ³ H]-thymine	[2- ¹⁴ C]-uracil	L-[U- ¹⁴ C]-leucine
0.05	25.1	-4.9	-17.6
0.10	36.1	-8.7	-6.9
0.20	48.4	0	32.2
0.50	87.9	74.8	82.9
1.00	96.6	98.7	90.1

^a Results are expressed as percentages of control values for preparations which lacked antibiotic and are based on the radioactivity found in TCA-precipitated material in 90 min.

FIG. 3. Absorption spectra of naphthyridinomycin (27 $\mu\text{g/ml}$) before (solid line) and after (dashed line) the addition of 1.0 mM DTT in 0.05 M Tris-hydrochloride (pH 7.9).

absorbance of the culture at 660 nm, *E. coli* LC462 growth was significantly reduced at naphthyridinomycin concentrations as low as 0.1 $\mu\text{g/ml}$ (data not shown). At higher concentrations, no change in the absorbance of the culture with time was observed. Counts of the viable colony-forming units at a concentration of 0.1 μg

naphthyridinomycin per ml indicated that viability decreased rapidly. Higher concentrations of naphthyridinomycin produced a dramatic reduction in viable colony-forming units, even in the zero-time samples. Although the test organism used in this study was different than that used in the previous study (12), our results indicated that naphthyridinomycin was effective in inhibiting bacterial growth at concentrations comparable to those reported previously.

Naphthyridinomycin is supposed to affect DNA synthesis more than RNA synthesis or protein synthesis at low antibiotic concentrations (11). Our results with *E. coli* LC462 confirm the previous finding (Table 2). DNA synthesis in *E. coli* LC462 was more severely affected at low antibiotic concentrations than RNA synthesis or protein synthesis. At a concentration of 0.2 μg of naphthyridinomycin per ml, DNA synthesis was inhibited 48.4%, protein synthesis was inhibited 32%, and RNA synthesis was not affected at all. The slight stimulatory effect of low naphthyridinomycin concentrations on RNA synthesis observed in this study was also observed in the previous study. Protein synthesis in *E. coli* LC462 in the presence of naphthyridinomycin was not affected at low antibiotic concentrations and was still less severely impaired compared with DNA synthesis in the presence of 0.2 μg of naphthyridinomycin per ml. These results confirm that the primary effect of naphthyridinomycin on bacterial cells is inhibition of DNA synthesis.

Effects of reducing reagents on naphthyridino-

TABLE 3. Binding of [^3H]naphthyridinomycin to DNA in the presence of reducing agents^a

Expt	Addition		% Of added ^3H bound to DNA	Ratio of naphthyridinomycin to DNA ^b
	Reducing agent	Concn (mM)		
1			0	0.0
2	DTT	1	0	0.0
3			7.1	0.009
4	DTT	0.01	11.9	0.016
5	DTT	0.1	21.2	0.028
6	DTT	1.0	26.9	0.036
7	Glutathione	1.0	10.7	0.014
8	Glutathione	10.0	17.2	0.023
9	L-Cysteine	1.0	8.2	0.011
10	L-Cysteine	10.0	15.1	0.020
11	Penicillamine	1.0	20.5	0.027
12	Penicillamine	10.0	30.0	0.040
13	Sodium borohydride	1.0	7.1	0.009
14	Sodium borohydride	10.0	0.45	0.0005
15	Sodium dithionite	1.0	1.0	0.0013
16	Sodium dithionite	10.0	3.6	0.005

^a [^3H]naphthyridinomycin and DNA were present at concentrations of 0.057 and 0.43 $\mu\text{mol/ml}$, respectively, in all reaction mixtures except those for experiments 1 and 2; DNA was not included in the reaction mixtures for experiments 1 and 2.

^b Calculated based on the radioactivity associated with the DNA and an $\epsilon_{260\text{nm}}$ value of 6,700 for calf thymus DNA.

TABLE 4. Binding of [³H]naphthyridinomycin to DNA, yeast RNA, and protein^a

Addition(s) ^b	% Bound to macromolecules	Ratio of naphthyridinomycin to base pairs
DNA	4.6	0.012
DNA, DTT (1.0 mM)	33.5	0.088
RNA	0.6	0.0015
RNA, DTT (1.0 mM)	1.1	0.0029
Protein	1.1	0.0
Protein, DTT (1.0 mM)	2.1	0.0

^a The protein used was bovine serine albumin. The concentrations used were as follows: [³H]naphthyridinomycin, 0.057 μ mol/ml (1,067 Bq/ml; 1 Bq = 1 dps); DNA, 0.215 μ mol/ml; yeast RNA, 0.215 μ mol/ml; and protein, 0.128 mg/ml.

^b The total volume of each incubation mixture was 5.0 ml.

mycin. The addition of naphthyridinomycin alone to buffered solutions of calf thymus DNA did not result in reproducible or dramatic changes in the UV absorption of the antibiotic (data not shown). This suggested that if naphthyridinomycin binds to DNA significantly, it might do so after activation. To test the possibility that naphthyridinomycin can undergo spectral changes similar to those of the activated saframycins (8, 9), various reducing reagents were added to the antibiotic in 0.05 M Tris buffer (pH 7.9), and the UV spectra were recorded immediately. Figure 3 shows that dithiothreitol (DTT) did cause a shift in the UV absorption maximum of the antibiotic from 270 to 287 nm. Of the potential reducing agents tested, DTT and penicillamine at concentrations of 1.0 mM were the best agents in causing this shift in absorption maximum. Cysteine caused the same change in the UV absorption maximum of naphthyridinomycin but required a final concentration of 10 mM to do so (data not shown). Glutathione, sodium borohydride, and sodium dithionite had no effect, even at high concentrations (10 mM).

Reactivity of [³H]naphthyridinomycin toward DNA. The reactivity of [³H]naphthyridinomycin toward calf thymus DNA was tested in the presence and in the absence of the potential reducing reagents to determine whether the spectral changes observed were significant. We believed that because of these spectral changes, the use of labeled naphthyridinomycin would cause the least degree of ambiguity in interpreting our results. As Table 3 shows, [³H]naphthyridinomycin was not retained in the dialysis bag in the absence of DNA (Table 3, experiments 1 and 2). Without a reducing agent (experiment 3), a small amount of antibiotic was bound to the

DNA. The addition of DTT markedly stimulated the binding of the antibiotic to DNA in a concentration-dependent fashion (experiments 4 through 6). DTT and penicillamine were about 10 times more effective than cysteine in activating naphthyridinomycin to bind to DNA. Borohydride and dithionite had no effect on binding and were actually inhibitory to binding at high concentrations. Although glutathione did not detectably change the UV maximum of the antibiotic, it was still able to stimulate antibiotic binding to the DNA.

Specificity of the binding of [³H]naphthyridinomycin to DNA. The affinity of [³H]naphthyridinomycin for other cellular macromolecules was tested. As Table 4 shows, [³H]naphthyridinomycin bound to DNA more efficiently than it bound to either RNA or protein. The addition of DTT to the incubation mixture greatly stimulated binding of naphthyridinomycin to DNA but had little effect on binding to RNA or protein. Additional studies with polydeoxyguanydic acid-polydeoxycytidylic acid and polydeoxyadenylic acid-polydeoxythymidylic acid (Table 5) demonstrated that [³H]naphthyridinomycin was specifically reactive toward the deoxyguanydic acid-deoxycytidylic acid base pairs of the DNA. This double-stranded polymer was a better substrate for naphthyridinomycin than calf thymus DNA, and virtually none of the antibiotic bound to polydeoxyadenylic acid-polydeoxythymidylic acid.

Effect of naphthyridinomycin on in vitro DNA synthesis and RNA synthesis. The binding of [³H]naphthyridinomycin to DNA would only be an interesting curiosity if the binding of the antibiotic did not affect template activity. Table 6 shows that naphthyridinomycin did have an appreciable effect on both DNA-dependent DNA synthesis and RNA synthesis. In the in vitro system used, naphthyridinomycin was more effective in inhibiting RNA synthesis than

TABLE 5. Binding of [³H]naphthyridinomycin to polydeoxyribonucleic acids in the presence of 1.0 mM DTT^a

Polydeoxyribonucleic acid ^b	Radioactivity bound (Bq/ml) ^c
Calf thymus DNA	68.3
poly(dG)-poly(dC)	169.3
poly(dA)-poly(dT)	0.6

^a The concentration of [³H]naphthyridinomycin used was 0.057 μ mol/ml (1,067 Bq/ml).

^b The concentration of DNA used was 1.25 absorbance units per ml. Abbreviations: poly(dG)-poly(dC), polydeoxyguanydic acid-polydeoxycytidylic acid; poly(dA)-poly(dT), polydeoxyadenylic acid-polydeoxythymidylic acid.

^c Total volume, 2.0 ml.

TABLE 6. Effects of naphthyridinomycin and 1.0 mM DTT on DNA-dependent synthesis and RNA synthesis^a

Naphthyridinomycin concn (M)	Inhibition of DNA synthesis (%)	Inhibition of RNA synthesis (%)
10 ⁻⁴	71	87
10 ⁻⁵	49	73
10 ⁻⁶	34	48

^a Naphthyridinomycin and DTT were preincubated with DNA for 30 min before enzymes were added. The synthesis of macromolecules was determined by counts in a TCA precipitate after 30 min.

DNA synthesis at high antibiotic concentrations. At very low concentrations, the syntheses of the two macromolecules were comparable. If DTT was omitted from the incubation medium, the highest concentration of naphthyridinomycin tested (10⁻⁴ M) inhibited DNA synthesis by only 47%, and RNA synthesis was inhibited by 52%.

DISCUSSION

The results presented above indicate that the antibiotic produced by *S. lusitanus* in our laboratory is the antibiotic previously reported to be naphthyridinomycin (10). The UV, infrared, and proton nuclear magnetic resonance spectra of this antibiotic, as well as its ruby red color, are consistent with the results of the previous study on the isolation of the antibiotic (10). The biological activity of the compound isolated in our study is also the same as to that reported for naphthyridinomycin (12). This antibiotic inhibits the growth of *E. coli* at low concentrations and at higher concentrations causes a dramatic decrease in cell viability. This inhibitory activity of naphthyridinomycin can be attributed to its ability to inhibit DNA synthesis in susceptible cells. A more substantial effect on RNA synthesis and protein synthesis was observed only at higher antibiotic concentrations.

Naphthyridinomycin is related structurally to the saframycins and inhibits DNA synthesis in a fashion similar to saframycin S (by binding to the DNA template [9]). The binding of naphthyridinomycin to DNA can also explain the three major effects of this antibiotic on susceptible cells observed in the previous study (12). In that study, the antibiotic was found to inhibit DNA synthesis, to require actively growing cells to be effective, and to be irreversible in its effects. The ability of the antibiotic to inhibit DNA synthesis is due to its affinity for deoxyguanylyl acid-deoxycytidylyl acid base pairs in DNA. Our in vitro study demonstrated that the naphthyridinomycin-DNA complex is a poor substrate for DNA-dependent polymerases. Our finding that

in vitro RNA synthesis was affected more than DNA synthesis at low antibiotic concentrations is not surprising. A number of DNA-reactive antibiotics have different effects on DNA synthesis and RNA synthesis, depending on the assay or test cells used (5). The requirement for metabolism in susceptible cells is most probably due to the necessity of converting naphthyridinomycin to its reactive form. Although sulfhydryl compounds do activate this antibiotic, naphthyridinomycin is more than likely activated by an enzymatic process in cells. This would be analogous to the activation of the mitomycins (14) observed in vitro and in vivo. The only discrepancy is the finding that glutathione can activate naphthyridinomycin without causing a change in its UV absorption maximum. This suggests that there might be two mechanisms for activation of this antibiotic. Finally, the irreversibility of the action of naphthyridinomycin can be explained by its strong binding to DNA. Precipitation of naphthyridinomycin-DNA complexes with ethanol did not release much antibiotic, although naphthyridinomycin is readily soluble in ethanol (data not shown). Therefore, the in vitro mechanism proposed accounts for the major in vivo results observed.

This study indicates that naphthyridinomycin is similar in its mechanism of action to saframycin S (9) and possibly to the pyrrolo(1,4)benzodiazepine antitumor antibiotics (6). All three groups of antibiotics contain a carbinolamine functionality. In the case of pyrrolo(1,4)benzodiazepine antibiotics, the carbinolamine functionality has been directly implicated in the binding site of these antibiotics to DNA (7). Naphthyridinomycin also contains a carbinolamine at a position analogous to the position of the carbinolamine in saframycin S. Based on results with other carbinolamine antibiotics, it would be reasonable to speculate that the carbinolamine of naphthyridinomycin is involved in the binding of this antibiotic to DNA. Reduction or activation of the antibiotic presumably could be a mechanism which allows naphthyridinomycin to interact better with its reactive site on the DNA, or this process might be required for stabilization of the adduct formed between the antibiotic and DNA.

Additional studies are under way to characterize the reaction of naphthyridinomycin with DNA and to determine the effects that this antibiotic has on the physical and chemical properties of DNA. Our results also suggest that naphthyridinomycin may possess antitumor activity, and this is currently being examined.

ACKNOWLEDGMENTS

Financial support for this work was provided by Public Health Service Biomedical Grant RR05738-09 from the Na-

tional Institutes of Health to the University of Utah College of Pharmacy and AI 16771. K.M.-H. was supported by Public Health Service Postdoctoral Traineeship CA09038 from the National Cancer Institute. The nuclear magnetic resonance data were provided by grant PCM-7922984 from the National Science Foundation to the Department of Medicinal Chemistry.

The technical assistance of Raissa Chacon and Roland Wong is also gratefully acknowledged.

LITERATURE CITED

1. Arai, T. 1979. The structures of novel antibiotics, saframycin B and C. *Tetrahedron Lett.* **25**:2355-2358.
2. Arai, T., K. Takahashi, K. Ishiguro, and A. Kubo. 1980. The structure of a novel antitumor antibiotic, saframycin A. *Experientia* **36**:1025-1026.
3. Arai, T., K. Takahashi, K. Ishiguro, and K. Yazawa. 1980. Increased production of saframycin A and isolation of saframycin S. *J. Antibiot.* **33**:951-960.
4. Burgess, R. R. 1969. A new method for the large scale purification of *Escherichia coli* deoxyribonucleic acid-dependent ribonucleic acid polymerase. *J. Biol. Chem.* **244**:6160-6167.
5. Goldberg, I. H., T. A. Beerman, and R. Poon. 1977. Antibiotics: nucleic acids as targets in chemotherapy, p. 427-456. In F. F. Becker (ed.), *Cancer: a comprehensive treatise*. Plenum Publishing Corp., New York.
6. Hurley, L. H., E. Gairola, and M. J. Zmijewski, Jr. 1977. Pyrrolo (1,4) benzodiazepine antitumor antibiotics: *in vitro* interaction of anthramycin, sibiromycin, and tomasmycin with DNA using specifically radiolabeled molecules. *Biochim. Biophys. Acta* **475**:521-535.
7. Hurley, L. H., and R. Petursek. 1981. Proposed structure of the anthramycin-DNA adduct. *Nature (London)* **282**:529-531.
8. Ishiguro, K., S. Sakimaya, K. Takahashi, and T. Arai. 1978. Mode of action of saframycin A, a novel heterocyclic quinone antibiotic. Inhibition of RNA synthesis *in vivo* and *in vitro*. *Biochemistry* **17**:2545-2550.
9. Ishiguro, K., K. Takahashi, K. Yazawa, S. Sakiyama, and T. Arai. 1981. Binding of saframycin A, a heterocyclic quinone anti-tumor antibiotic to DNA as revealed by the use of the antibiotic labeled with [¹⁴C]tyrosine or [¹⁴C]cyanide. *J. Biol. Chem.* **256**:2162-2167.
10. Kluepfel, D., H. A. Baker, G. Plattoni, S. N. Sehgal, A. Sidorowicz, K. Singh, and C. Vezina. 1975. Naphthyridinomycin, a new broad-spectrum antibiotic. *J. Antibiot.* **28**:497-502.
11. Lehman, I. R. 1963. DNA synthesis (bacterial). *Methods Enzymol.* **6**:34-39.
12. Singh, K., S. Sun, and D. Kluepfel. 1976. Mechanism of action of naphthyridinomycin on *Escherichia coli*. *Dev. Ind. Microbiol.* **17**:209-221.
13. Sygusch, J., F. Brisse, S. Hanessian, and D. Kluepfel. 1974. The molecular structure of naphthyridinomycin, a broad-spectrum antibiotic. *Tetrahedron Lett.* **46**:4021-4023.
14. Tomasz, M., C. M. Mercado, J. Olson, and N. Chatterjee. 1974. The mode of interaction of mitomycin C with deoxyribonucleic acid and other polynucleotides *in vitro*. *Biochemistry* **13**:4878-4887.