Structure-Activity Relationship of Daunorubicin and Its Peptide Derivatives

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The synthesis of potentially specific antitumor peptide derivatives of daunorubicin is presented. The interaction specificities of the drugs with nucleic acids have been examined via stop-flow kinetics as well as the inhibition of DNA template activity. It is found that the biological activity of the daunorubicin derivatives against the mouse P388 tumor is directly proportional to RNA polymerase inhibition and inversely proportional to the rate of dissociation of the DNA complex. It is concluded that the biological efficacy of drugs which act at the replicative and transcriptional level may be estimated by the more rapid in vitro techniques provided that problems of permeability, solubility, stability, etc., in vivo are not encountered.

Daunorubicin,[2,3] a glycosidic anthracycline antibiotic from *Streptomyces peucetius*,[4] is being used in the treatment of acute leukemia and solid tumors in man.[5,6] The biological activity seems to be due to complex formation with the DNA of deoxyribonucleoprotein.[4] In vivo, daunorubicin inhibits both RNA and DNA synthesis[7,8] and, in vitro, DNA- and RNA-dependent polymerases.[7-9,28,36] Unfortunately, the drug as well as many of its derivatives exhibits a deleterious effect on humans; e.g., congestive heart failures have been observed in a number of patients who have received a cumulative dose of 20 mg/kg or more.[10-15]

In order to increase the chemotherapeutic value of the daunorubicin molecule, attempts have been made to utilize (i) the higher endocytic activity of tumor as compared to normal cells[16] and (ii) selective recognition of antitumor specific antibodies covalently labeled with daunorubicin.[17,18] In the present approach, the difference in the pH of the cytoplasm of tumor (pH 6.7) and normal cells (pH 7.5)[19,20] is utilized in order to achieve selective biological activity of the daunorubicin molecule. The syntheses of the amino acid amides **1-3** have been undertaken since it is well known that the pK_a of amino groups in such compounds is approximately 1.5-2.0 pH units lower than in amino acids and/or simple alkylamines.[21] For example, the peptide derivatives of daunorubicin, **1-3**, are observed to have a pK_a of 7.4, whereas the parent compound exhibits a pK_a of 8.99 for the amino group of the sugar moiety. It is reasoned, therefore, that the amides **1-3** would exist primarily in the cationic form in tumor cells and in the neutral form in normal cells. Since (i) the biological activity of daunorubicin is due to complex formation with the DNA of chromatin and (ii) it is known that the cationic form of the drug is required for effective binding to DNA, it is therefore reasonable to believe that the peptide derivatives **1-3** may exhibit a greater cytotoxic activity against tumor as compared to normal cells. The above arguments naturally presuppose that the peptide derivatives retain their integrity and do not undergo in vivo hydrolysis by peptidases.

In this paper we report the syntheses of the potentially specific antitumor peptide derivatives of daunorubicin, **1-3**. In addition, data on the interaction specificities of the derivatives with nucleic acids as well as their biological activity against the mouse P388 lymphocytic tumor are presented. It is found that the in vivo activity of **1-3** is (i) directly proportional to the in vitro inhibition of DNA transcription and (ii) inversely proportional to the rate of dissociation of the DNA complex.

Results

The syntheses of the amino acid derivatives of daunorubicin, **1-3**, which are found to proceed smoothly and in good yield employ the mixed anhydride procedure of Anderson et al.[22] as indicated in Chart I. The authenticity and purity of the derivatives were checked by elemental analysis, thin-layer chromatography, Fourier transform proton magnetic resonance, absorption spectroscopy, and circular dichroism. Table I lists the chemical shifts and multiplicity of the proton signals of daunorubicin and derivatives **1-3** in $CDCl_3$. Absorption and circular dichroism data are given in Table II.

Table I. Chemical Shifts, δ (Hz), from Me_4S_6 of the ^1H NMR Signals of Daunorubicin (D) and Peptide Derivatives **1-3** in $CDCl_3$[a]

Proton signal[b]	δ, Hz (multiplicity)[c]			
	D	D-Me_2Gly	D-Me_2-GlyGly	D-Pr_2Gly
H_1	802 (dd)	805 (dd)	803 (dd)	806 (dd)
H_2	778 (t)	779 (t)	777 (t)	780 (t)
H_3	738 (dd)	738 (dd)	736 (dd)	739 (dd)
$H_{1'}$	550 (m)	554 (m)	551 (m)	554 (m)
H_7	529 (m)	510 (m)	526 (m)	528 (m)
OCH_3	408 (s)	409 (s)	407 (s)	410 (s)
$H_{4'}$	347 (m)	371 (m)	365 (m)	371 (m)
H_{10e}	325 (d)	328 (d)	324 (d)	330 (d)
H_{10a}	293 (d)	293 (d)	290 (d)	293 (d)
$COCH_3$	240 (s)	242 (s)	241 (s)	242 (s)
$CH_3(5')$	134 (d)	130 (d)	128 (d)	131 (d)
$DCOCH_2N$		291 (s)	299 (s)	300 (s)
$DCOCH_2$-NHCO-CH_2N-$(CH_3)_2$			217	
$DCOCH_2$-$N(CH_3)_2$		227 (s)	230 (s)	
$DCOCH_2$-$N(CH_2$-CH_2-$CH_3)_2$				145 (t)
$DCOCH_2$-$N(CH_2$-CH_2-$CH_3)_2$				90 (t)

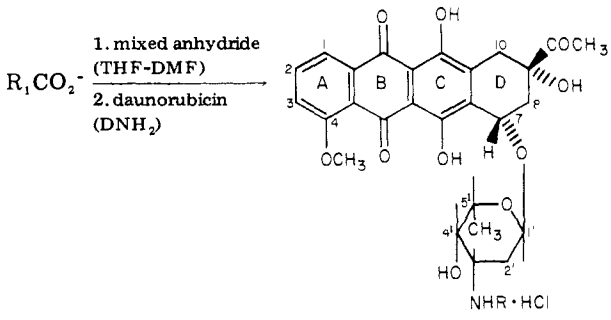
[a] The hydrochloride salts of D and **1-3** in D_2O were brought to pH 10.0 with borate buffer and the neutral amine form was extracted in $CDCl_3$. The deuterated solvent was evaporated and the product was dried over P_2O_5 in a desiccator. ^1H NMR spectra of the D-exchanged product were taken in $CDCl_3$ using the Varian XL-100-25 spectrometer in the FT mode. [b] Only the clearly discernible ^1H NMR signals are reported. H_{8a}, H_{8e}, $H_{2'}$, $H_{3'}$, and $H_{5'}$ are observed to be complex overlapping multiplets. [c] Multiplicity notations as follows: s (singlet), d (doublet), dd (doublets of doublets), m (multiplet), t (triplet).

The effect of daunorubicin and compounds **1-3** on the DNA-dependent *Escherichia coli* RNA polymerase is shown in Figure 1. It is noted that DNA transcription (as measured by [5-^{3}H]-UMP incorporation into RNA) is inhibited to a different extent by the anthracycline drugs,

Table II. Absorption and Circular Dichroism Characteristics of Daunorubicin and Peptide Derivatives 1-3 in 10 mM 2-(*N*-Morpholino)ethanesulfonic Acid Buffer (MES), pH 6.2, and 5 mM Na^+ at 25°C

Compound	Absorption		Circular dichroism			
	λ_{max}	ϵ_{max}	λ_{P_1}	$[\theta]_{P_1}$	λ_{P_2}	$[\theta]_{P_2}$
Daunorubicin (D)	477	11 960	468	4.2×10^3	346	8.4×10^3
D-Me_2Gly (1)	477	11 730	466	5.9×10^3	341	8.2×10^3
D-Me_2GlyGly (2)	477	11 380	467	6.1×10^3	342	8.6×10^3
D-Pr_2Gly (3)	477	11 560	465	6.7×10^3	343	9.0×10^3

Chart I



	R	NSC	pK_a
daunorubicin (D)	H	82151	8.99 ± 0.03
D-Me_2Gly (1)	$COCH_2H(CH_3)_2$	246104	7.35
D-Me_2GlyGly (2)	$COCH_2NHCOCH_2N(CH_3)_2$	246132	7.39
D-Pr_2Gly (3)	$COCH_2N(C_3H_7)_2$	246105	7.20[a]

[a] In 20% EtOH-H_2O. The D-Pr_2Gly, 3, is insoluble in H_2O at pH >7.0.

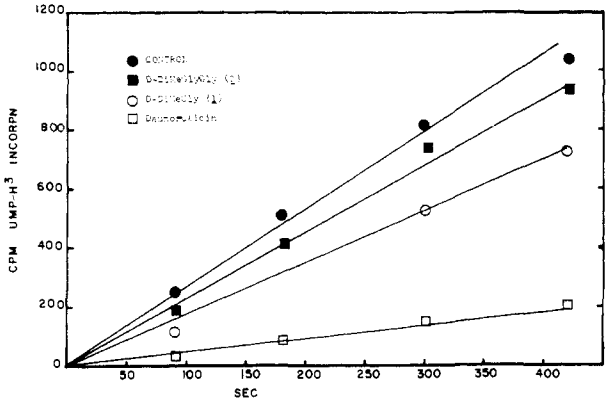


Figure 1. The effect of daunorubicin and peptide derivatives 1 and 2 on the kinetics of incorporation of [^{3}H]-UMP into RNA by *E. coli* RNA polymerase. Calf thymus DNA (0.12 mM P/l.) and the drugs at 0.12 mM were used.

i.e., daunorubicin > D-Me2Gly > D-Me2GlyGly. The effect of the Pr2Gly derivative **3** on the kinetics of the transcription process could not be determined due to solubility problems.

The first-order rate plots for the dissociation of the salmon sperm DNA complex of daunorubicin and derivatives **1-3** are shown in Figure 2 and the results are summarized in Table III. A stop-flow technique (similar to the method described by Muller and Crothers[23]) is used to monitor the hyperchromic change accompanying the dissociation of the DNA–D complex caused by the sequestering agent, sodium dodecyl sulfate (SDS), according to the eq 1. The dissociation reaction is found to be first

$$\text{DNA-D} \xrightarrow{k} \text{DNA} + \text{D}$$

$$\text{D} + \text{SDS} \xrightarrow{k_2} \text{SDS-D} \qquad (1)$$

order (to at least 2.5 half-lives) in DNA complex and is

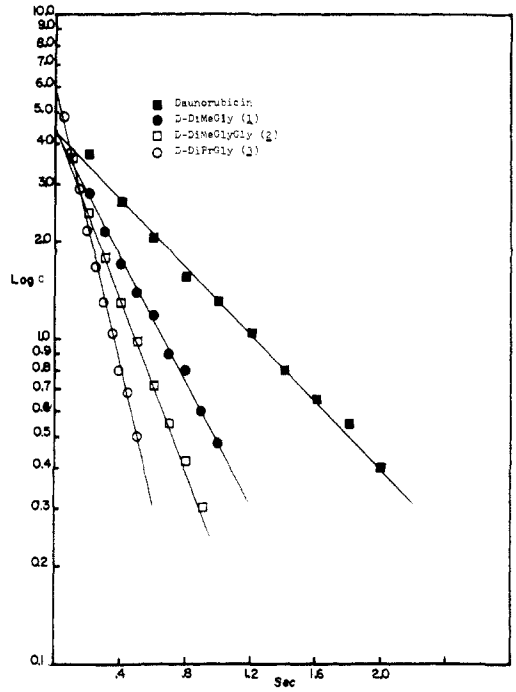


Figure 2. The first-order rate plots for the dissociation of the DNA complexes of daunorubicin in MES buffer (pH 6.2 and 5 mM Na^+) at 15°C (see Table III for details).

Table III. Effect of pH and Ionic Strength on the First-Order Dissociation Rates of the DNA-Daunorubicin and **1-3** Complexes[a]

Salmon sperm DNA complex	k, sec^{-1}				
	pH 6.2		pH 7.8		
	5 mM Na^+	21 mM Na^+	1 mM Na^+	21 mM Na^+	201 mM Na^+
Daunorubicin	0.98	0.98	1.37	1.57	2.88
D-Me_2Gly	2.32	3.51	2.70	4.80	11.70
D-Me_2GlyGly	2.83	4.72	3.09	5.92	15.40
D-Pr_2Gly	5.14	6.63	2.96	8.22	17.03

[a] Kinetics of dissociation were measured at 480 nm by stop-flow techniques at 15°C using 0.2% SDS in either 10 mM MES buffer (pH 6.2) (5 mM Na^+) or 1 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) buffer (PIPES) (pH 7.8, 1 mM Na^+) and at various Na^+ concentrations (NaCl). Salmon sperm DNA-X complex was prepared in the same buffers (in the absence of SDS) at 6 μM concentration of the drug and 0.36 mM DNA P/l. The reactions are found to be first order to at least 2.5 half-lives. Increasing the concentration of SDS (0.2-1.0%) and/or the concentration of DNA (base pair/drug = 3-30) has minimal effect on the observed rate constants, i.e., ±3%.

independent of SDS concentration and/or the concentration of DNA (at base pair/drug = 3–30). The results are found to be consistent with eq 1 whereby the first-order rate constant, k, of the dissociation process is the rate-controlling step and the bimolecular sequestering process, k_2, is diffusion controlled. For this reason, we have been

able to measure the first-order rate constant for the dissociation of DNA–D and DNA–1–3 as a function of ionic strength and pH. The following observations are noted (see Table III). (i) The effect of increasing pH from 6.2 to 7.8 (at constant ionic strength, e.g., 21 mM Na^+) enhances the dissociation rate constants by a factor of 1.60, 1.37, 1.25, and 1.24 for the DNA complexes of daunorubicin (D), **1**, **2**, and **3**, respectively. (ii) The effect of increasing ionic strength (at constant pH, e.g., pH 7.8) from 1 to 201 mM Na^+ enhances the dissociation rates by a factor of 2.10, 4.33, 4.98, and 5.75 for the DNA–D, **1**, **2**, and **3** complexes, respectively. (iii) At pH 7.8 and 201 mM Na^+ (resembling physiological conditions) the DNA–1–3 complexes dissociate at rates of 4.06, 5.35, and 5.90 (for **1**, **2**, and **3**, respectively) faster than the DNA–D complex.

Preliminary screening by the Drug Research and Development Branch of the National Cancer Institute of derivatives **1–3** using 0.25–10.0 mg/kg daily dosage in the treatment of the mouse P388 lymphocytic leukemia tumor indicates the following order of decreasing biological activity, daunorubicin (D) > **1** > **2**. Compound **3**, the Pr_2Gly derivative, is found to be devoid of activity. It should be noted, however, that the latter compound is insoluble in water at pH >7.0 and, therefore, the vehicle and site of administration of the drug (water and peritoneal injection) may have rendered it ineffective—although this may not be the only explanation (see below).

Discussion

Considerable interest has and is presently being devoted by various laboratories to the synthesis of derivatives of daunorubicin in order to improve the chemotherapeutic index of the anthracycline drugs.[24-27] Our preliminary approach has been centered on the modification of the amino group of the sugar moiety of daunorubicin and future efforts will also consider the effect of modifications of the A and D ring substituents (see Chart I) as well as the sugar moiety.

It is well known that daunorubicin exhibits its cytotoxic properties by binding to DNA, thus leading to inhibition of the replicative and transcription processes.[7-9,28] Therefore, in vitro assays of the (i) rate of dissociation of DNA complexes as well as (ii) the inhibition of DNA-dependent polymerases are expected to provide a rapid estimate of the biological activity of daunorubicin and its derivatives. Moreover, important information is obtained at the molecular level which may be relevant to the proper design of chemotherapeutically more effective drugs. It is recognized, of course, that the in vivo situation is considerably more complicated due to problems of permeability, solubility, competitive binding to proteins and/or lipids, stability of the derivatives, etc. Nonetheless, our preliminary in vitro studies on the peptide derivatives **1–3** indicate that these molecules (i) dissociate from the DNA complex in the order **3** > **2** > **1** > daunorubicin (D) (see Table III) and (ii) inhibit the DNA-dependent *E. coli* RNA polymerase in the *inverse* order, i.e., D > **1** > **2** (Figure 1). The above results are consistent with the relative in vivo biological activity on the mouse P388 lymphocytic tumor, i.e., D > **1** > **2** (**3** is biologically inactive). Similar finding, i.e., inverse relationship of the dissociation rate with respect to template inhibition and biological activity, has also been observed by Muller and Crothers[23] for the actinomycin D and related systems.

At the molecular level, it is likely that the larger tertiary ammonium cations, i.e., $-^+NH(CH_3)_2$ and $-^+NH(C_3H_7)_2$ of the amino acid side chains, in the derivatives **1–3** are responsible for lowering the H bonding and electrostatic interactions with the DNA phosphate groups presumably due to steric factors. Such effects can account for the faster dissociation rates of **1–3** as compared to the parent molecule, daunorubicin, at any given ionic strength and pH (see Table III). Moreover, evidence for a decreased electrostatic interaction between DNA and **1–3** as compared to daunorubicin is noted. For example, at pH 7.8 the rate of dissociation of the DNA–X complexes is found to increase by a factor of 2.10, 4.33, 4.98, and 5.75 for DNA–D, **1**, **2**, and **3**, respectively, as the Na^+ concentration is increased from 1 to 201 mM. The greater electroscreening effects of the Na^+ ion have been previously noted for the interactions of DNA with diamines,[29] polyamines,[30] and steroidal amines[31,32] as the ammonium salts of the latter are progressively substituted, i.e., primary < secondary < tertiary < quaternary. Efforts are currently in progress to synthesize *N*-acyl derivatives of daunorubicin whereby the side chains contain primary ammonium group(s) as well as H-bond donors and acceptors in the hope of producing compounds which will dissociate less rapidly from the DNA complex.

Experimental Section

The syntheses of the peptide derivatives 1–3 were carried out by the mixed anhydride procedure of Anderson et al.[22] as follows.

***N,N*-Dimethylglycyldaunorubicin (1).** To 21 mg (0.21 mmol) of *N,N*-dimethylglycine (synthesized according to the reductive alkylation procedure of Bowman and Stroud[33]) in 25 ml of dry dimethylformamide (DMF) at −10°C were added 21 mg (0.21 mmol) of triethylamine and 34 mg (0.25 mmol) of isobutyl chloroformate and the solution was stirred for 20 min under dry N_2 at −10°C. Daunorubicin hydrochloride (gift from Rhone-Poulenc) was added (100 mg, 0.17 mmol) together with 17 mg of triethylamine (0.17 mmol) and the reaction mixture was maintained at −10°C for 1 h and then allowed to come to room temperature. The solvent was evaporated by a steady stream of dry N_2 (overnight) and the solid material was dissolved in 30 ml of chloroform and extracted four times with equal volumes of a saturated solution of sodium bicarbonate and four times with diionized water. The chloroform was then evaporated and the product was dissolved in 5 ml of ethanol and 0.2 mM HCl was added. TLC on silica gel plates (10% methanol–chloroform) indicated a mixture of three compounds, i.e., daunorubicin (R_f 0.17), compound 1 (R_f 0.68), and the isobutylurethane adduct of daunorubicin (R_f 0.72). Quantitative separation of the three compounds was affected by silica gel column chromatography (0.5 × 30 cm) using a 5% methanol–$CHCl_3$ mixture. The product, 1, was obtained in 63% yield after recrystallization of the intermediate fraction from an ethanol–ethyl acetate solvent mixture. Anal. ($C_{31}H_{36}N_2O_{11}\cdot HCl\cdot H_2O$) C, H.

***N,N*-Dimethylglycylglycyldaunorubicin (2).** A similar synthetic procedure as described for 1 was used to couple *N,N*-dimethylglycylglycine (prepared according to the procedure of Bowman and Stroud[33]) with daunorubicin. The products were separated by silica gel column chromatography (10% methanol–chloroform as eluent) and recrystallized from an ethanol–ethyl acetate mixture to yield 67 mg (55% yield) of **2**. Anal. ($C_{33}H_{39}N_3O_{12}\cdot HCl$) C, H.

***N,N*-Dipropylglycyldaunorubicin (3).** *N,N*-Dipropylglycine[33] was coupled to daunorubicin according to the procedure described previously for the synthesis of 1. The products were separated by silica gel chromatography (2% methanol–chloroform as eluent) and the major fraction was recrystallized from a methanol–ether mixture to yield 78 mg (63% yield) of **3**. Anal. ($C_{35}H_{45}O_{11}N_2\cdot HCl\cdot H_2O$) C, H.

The authenticity and purity of the peptide derivatives 1–3 were also checked (i) by proton magnetic resonance (in the neutral form in $CDCl_3$; see Table I), (ii) by uv–visible and circular dichroism spectroscopy (Table II), (iii) by molecular weight determination using a titrimetric method, and (iv) by TLC on silica gel.

^1H NMR spectra were recorded at 34°C on a Varian XL-100-15 spectrometer with a Nicolet technology corporation FT accessory in $CDCl_3$ using tetramethylsilane (Me_4Si) as the internal standard. Absorption spectra were recorded on a Cary 15 spectrometer and circular dichroism spectra were recorded on a Jasco J-20 spec-

tropolarimeter at 25°C. Molecular weight and pK_a values were obtained potentiometricly at ambient temperature (25 ± 1°C) using a Radiometer titrigraph automatic titrator equipped with a micro-cell assembly. Stop-flow kinetics were carried out using a Durrum-Gibson apparatus (Durrum Instrument Corp.) thermostated with a Lauda K-2/R constant temperature circulator (see Table III for details). The kinetics of the DNA-dependent *E. coli* RNA polymerase reaction was followed by the incorporation of [5-^{3}H]-UMP into TCA-insoluble RNA according to the procedure of Preston et al.[34] Radioactivity was counted on an LS-133 liquid scintillation counter (Beckmann Instrument Co.).

Salmon sperm and calf thymus DNA were obtained from Worthington Corp. and found to be free of any detectable protein contaminants. Stock solutions of polymers were made in 10 mM 2-(*N*-morpholino)ethanesulfonic acid buffer (MES), pH 6.2, 5 mM Na^+, and stored at 0°C. The stock solutions were removed and diluted in the various buffers as indicated in the tables and figures. *E. coli* RNA polymerase was extensively purified through the step involving high salt glycerol gradient centrifugation according to the method of Burgess.[35] The activity was precipitated with 1.5 vol of saturated $(NH_4)_2SO_4$, collected by centrifugation at 3000g for 30 min, and suspended in 10 vol of buffer [10 mM Tris–HCl, pH 7.9, 10 mM $MgCl_2$, 100 mM KCl, 0.1 mM dithiothreitol, 0.1 mM EDTA, and 50% (v/v) glycerol]. The specific enzyme activity was determined by the incorporation of ^{3}H into the acid-insoluble product at 37°C using [5-^{3}H]-UTP as substrate was 19.7 nmol/min/mg of protein. Conditions for the assay of RNA polymerase were similar to that described by Preston et al.[34] The final reaction mixture contained 56 mM Tris–HCl, pH 7.8, 1.6 mM $MnCl_2$, 150 mM KCl, 1.6 mM β-mercaptoethanol, 0.6 mM each of GTP, ATP, and CTP (Sigma Chemicals), 0.15 mM [5-^{3}H]-UTP (53.3 Ci/mol), and 0.12 mM P/l. of native calf thymus DNA. *E. coli* RNA polymerase was present at 87 units/ml (1 unit is the amount of enzyme which will incorporate 1 μmol of UMP/min at 37°C). All components in the above mixture were brought to 37°C and the reaction was initiated by the addition of the polymerase. Samples of 100 μl were withdrawn at various intervals and immediately placed into a test tube containing 100 μl of a solution of 5 mM UTP, 0.1 M $Na_4P_2O_7$, 2 mg/ml of RNA (Torula yeast, Sigma), and 2 mg/ml of bovine serum albumin (Baker Chemicals). After 10–20 min, 1 ml of cold 10% TCA was added to each tube and the solution was allowed to incubate at 0°C for an additional 30 min. The mixture was then filtered on Whatman GF/C fiber disks which had been prewashed with 5 ml of cold 10% TCA solution. Each disk was then washed three times with 5–10 ml of TCA, three times with 5–10 ml of an ether–ethanol solution (3:1), and three times with 5–10 ml of ether, dried at 80°C, placed in scintillation vials containing 5 ml of toluene, 0.4% 2,5-diphenyloxazole, 0.01% 1,4-di[2-(5-phenyloxazolyl)]benzene, and counted for 1 h in a Beckman LS-133 liquid scintillation counter.

It should be noted that Hartman et al.[7] and Zunino et al.[28] have found in the presence of 0.1 mM calf thymus DNA and 0.05 mM daunorubicin, the *E. coli* RNA polymerase was 100% inhibited. In the present studies the observed inhibition is found to be approximately 90% under conditions utilizing 0.12 mM DNA P/l. and 0.12 mM daunorubicin (Figure 1). The reason for the discrepancy is the fact that the enzymatic assays were carried out at higher ionic strength ($\mu \simeq 200$ mM) in the present studies as compared to that reported in the literature ($\mu \simeq 20$ mM).[7,28] This was done in order to relate the in vivo biological activity of the drugs to the in vitro inhibition of the polymerase. As a consequence, the expected lower binding of daunorubicin to DNA at high ionic strength[7] would account for the observed data.

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References and Notes

(1) (a) On leave from Georgia State University, 1974–1975; (b) undergraduate research participants, University of Florida; (c) NIH Career Development Fellow, 1970–1975.
(2) (a) F. Arcamone, G. Franceschi, P. Orezzi, and S. Renco, *Tetrahedron Lett.*, **30**, 3349 (1968); (b) F. Arcamone, G. Cassinelli, G. Franceschi, P. Orezzi, and R. Mondelli, *ibid.*, **30**, 3353 (1968).
(3) R. Anguili, F. Arcamone, E. Foresti, N. W. Isaacs, O. Kennard, W. D. S. Motherwell, L. Riva di Sanseverino, and D. L. Wampler, *Nature (London), New Biol.*, **234**, 78 (1971).
(4) A. DiMarco, N. Gaetani, P. Orezzi, B. Scarpinato, R. Silverstrini, M. Soldati, T. Dasdia, and L. Valentini, *Nature (London)*, **201**, 706 (1964).
(5) C. Tan, H. Tasaka, K. Yu, M. L. Murphey, and D. A. Karnofoky, *Cancer*, **20**, 333 (1967).
(6) M. Boiron, C. Jacquillar, M. Weil, J. Tanzer, D. Levy, G. Sultan, and J. Bernard, *Lancet*, 330 (1969).
(7) G. Hartmann, G. Goller, K. Koschel, W. Kersten, and H. Kersten, *Biochem.*, **341**, 126 (1964).
(8) A. Theologides, J. W. Yarbro, and B. J. Kennedy, *Cancer*, **21**, 16 (1968).
(9) D. C. Ward, E. Reich, and I. H. Goldberg, *Science*, **149**, 1259 (1965).
(10) B. Smith, *Br. Heart J.*, **31**, 607 (1969).
(11) M. M. Raskin, M. G. Rajurkar, and D. H. Altman, *Am. J. Roentgenol.*, **118**, 68 (1973).
(12) L. M. Buja, V. J. Ferrans, R. J. Mayer, W. C. Roberts, and E. S. Henderson, *Cancer*, 771 (1973).
(13) J. F. Halazun, H. R. Wagner, J. F. Gaeta, and L. F. Sinks, *Cancer*, 545 (1974).
(14) R. H. Blum, M. A. Friedman, and S. K. Carter, Clinical Brochure (NSC-123127), National Cancer Institute, 1972.
(15) R. B. Livingston and S. K. Carter, *Chemother. Fact Sheet*, 1 (1970).
(16) A. Trouet, D. Deprez-De Campeneere, and C. De Duve, *Nature (London), New Biol.*, **239**, 110 (1972).
(17) E. Hurwitz, R. Levy, R. Maron, M. Wilchek, R. Arnon, and M. Sela, *Cancer Res.*, **35**, 1175 (1975).
(18) R. Levy, E. Hurwitz, R. Maron, R. Arnon, and M. Sela, *Cancer Res.*, **35**, 1182 (1975).
(19) W. C. J. Ross, *Biochem. Pharmacol.*, **8**, 235 (1961).
(20) A. A. Sinkula and S. H. Yalkowsky, *J. Pharm. Sci.*, **64**, 181 (1975).
(21) H. A. Sober, "Handbook of Biochemistry", 2nd ed, Chemical Rubber Publishing Co., Cleveland, Ohio, 1970.
(22) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Am. Chem. Soc.*, **89**, 5012 (1967).
(23) W. Muller and D. M. Crothers, *J. Mol. Biol.*, **35**, 251 (1968).
(24) K. Yamamoto, E. Acton, and D. W. Henry, *J. Med. Chem.*, **15**, 872 (1972).
(25) D. W. Henry, *Cancer Chemother. Rep., Part 2*, **4**, 5 (1974).
(26) L. Lenaz, A. Necco, T. Dasdia, and A. DiMarco, *Cancer Chemother. Rep., Part 1*, **58**, 769 (1974).
(27) G. Jolles, Rhone-Poulenc Research Laboratories, Paris, France, personal communication, 1975.
(28) F. Zunino, A. DiMarco, A. Zaccara, and G. Juoni, *Chem.-Biol. Interact.*, **9**, 25 (1974).
(29) E. J. Gabbay, *Biopolymers*, **5**, 727 (1967).
(30) R. Glaser and E. J. Gabbay, *Biopolymers*, **6**, 241 (1968).
(31) E. J. Gabbay, B. L. Gaffney, R. Glaser, and D. Z. Denney, *Chem. Commun.*, 1507 (1969).
(32) E. J. Gabbay and R. Glaser, *Biochemistry*, **10**, 1665 (1971).
(33) R. E. Bowman and H. H. Stroud, *J. Chem. Soc.*, 1342 (1950).
(34) J. F. Preston, H. S. Stark, and J. W. Kimbrough, *Lloydia*, **38**, 153 (1975).
(35) R. R. Burgess, *J. Biol. Chem.*, **244**, 6160 (1969).
(36) A. di Marco and F. Acramone, *Arzneim.-Forsch.*, **25**, 368 (1975).