The urinary excretion patterns of nadolol (I) and nadolol-d_9 (II) are summarized in Tables IV and V. Depending on the drug form, subject, and leg, 8–20% (mean 12%) and 7–17% (mean 10%) of the respective doses of I and II are excreted in 48 h. Although not entirely unexpected, the deuterated solution formulation (II) has lower bioavailability than either the solution or tablet formulations of I. From the statistical criteria applied to absolute bioavailability data, the deuterated solution formulation is marginally bioequivalent, differing by <20% from the nondeuterated tablet and solution formulations. The tablet and solution formulations of I are bioequivalent, on the other hand, and the urinary excretion values also parallel these observations.

The use of the nonadeuterated analogue was based on the availability of intermediates. Apparently extensive deuterium labeling in the side chain resulted in reduced absorption and lower bioavailability. While it might have been more appropriate to have used ^{13}C- and ^{15}N-labeled nadolol to reduce the isotope effects, the deuterated form yielded reliable results which paralleled those of the nonlabeled forms of I.

The mean serum values of the area under the bioavailability curve can be calculated from the absolute areas in the conventional way (18)[21] or from the relative area, defined as absolute area the formulation of I divided by the value for the coadministered II (Table VI). From the absolute area, seven to eight subjects would be required as the minimum number to demonstrate bioequivalence, while only four to five subjects would be required to show the bioequivalence using coadministration[21]. Increasing this number to six ensures staying within the confidence of establishing bioequivalence. The use of the relative parameter is even more striking in the evaluation of the urinary excretion data, which required a minimum of 10 subjects in the normal approach and either 2 or 4 subjects using relative bioavailability (Table VII).

For studies employing analytical methods with reasonable precision, the limitation in bioavailability studies continues to be in the variability of the disposition by the subjects. In these instances, the use of coadministration represents the optimum method of generating a reliable data set with a minimum of subjects. Rapidly processed, an early evaluation of the bioavailability of a new dosage form can be made. It also takes the greatest advantage of the MS selective capability. Finally, the introduction of new low-priced high-performance EI mass selective detectors should result in a wider acceptance of coadministration relative bioavailability and absolute bioavailability MS evaluations.

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A New Series of Reductive Amination Derivatives of Daunorubicin: Syntheses, Partition Coefficients, and DNA Binding

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Abstract □ A series of daunorubicin derivatives were prepared by sodium cyanoborohydride reductive amination of daunorubicin with appropriate amines. All derivatives were found to bind quite strongly to DNA and viscosity increases with linear DNA indicated that each formed an intercalation complex. A range of octanol-aqueous buffer partition coefficients was obtained, around the values of daunorubicin and dauxorubicin hydrochloride, by varying the character of the starting amine. All monoamine derivatives had activity against P388 leukemia in mice which was similar to that of daunorubicin. A diamine derivative had reduced activity against P388. Several anthracyclines administered as DNA complexes had similar activity against P388 but significantly reduced toxicity compared to the uncomplexed compounds. For anthracyclines which bind strongly to DNA, optimum activity against P388 leukemia in mice seems to be centered on compounds with octanol-buffer partition coefficients in the range of 0.5–0.8.

Keyphrases □ Anthracyclines—new derivatives, antineoplastic agents □ Toxicity—reduced cardiotoxicity, anthracyclines

The glycosidic anthracycline antibiotics daunorubicin (I) and dauxorubicin hydrochloride (II), are being clinically used as antineoplastic agents (1–8). Unfortunately, these drugs are severely limited by their dose related cardiotoxicity (1–5). Although the molecular basis of the cardiotoxicity is not clear, two proposed mechanisms are widely accepted. First, the anthracyclines are reduced to semiquinone free radicals and, subsequently, generate oxygen radicals which cause membrane lipid peroxidation and DNA strand scission (9–11). Second, the chemically labile glycosidic bonds of I and II are cleaved by glycosidases to form the respective aglycons during *in vivo* metabolism (1–4, 9, 12). The water insoluble aglycons accu-

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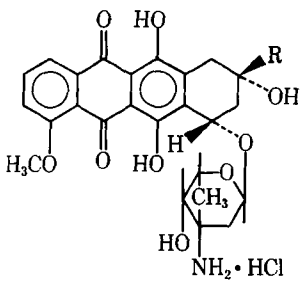
mulate in heart tissue. The anthracyclines are no doubt capable of multiple mechanisms of action at several biological sites. Their antitumor activity is believed to be at least partially due to their ability to bind *in vivo* to DNA of nuclear deoxyribonucleoprotein (1-8). The results of X-ray diffraction and model building studies have shown that there is a specific complex formed between the anthracyclines and DNA (7, 8). As part of a continuing effort (13, 14) to prepare derivatives of I and II with higher specificity, reduced toxicity, and also to develop *in vitro* methods for biological activity evaluation, we report the synthesis and biological effects of a new series of anthracycline amine derivatives IV–VII. The results of *in vivo* testing for the DNA complexes with some of the new analogues are also presented.

Since bulky substituents at C-13, such as rubidazone, III, maintain high antineoplastic activity (1), it is reasonable to expect that anthracycline analogues at this position would be active. In an attempt to reduce the accumulation of the aglycons in heart tissue, their water solubility was increased by introducing charged amino derivatives at C-13. It should be noted that these cationic groups could also potentially enhance binding affinity to DNA. In addition, varying the hydrophobic character of the substituents on C-13 was used to vary the partition coefficient of these C-13 derivatives.



I, R = $COCH_3$ (Daunorubicin)
II, R = $COCH_2OH$ (Dauxorubicin)
III, R = $C(NNHCOC_6H_5)CH_3$ (Rubidazone)
IV, R = $CH(NHCH_3{\cdot}HCl)CH_3$
V, R = $CH(NH(CH_2)_3CH_3{\cdot}HCl)CH_3$
VI, R = $CH(NH(CH_2)_3OH{\cdot}HCl)CH_3$
VII, R = $CH(NH(CH_2)_3NH_2(CH_3)_2{\cdot}2HCl)CH_3$
VIII, R = $CH(OH)CH_3$ (Daunorubicinol)

EXPERIMENTAL SECTION[1]

(7*S*,9*S*)-7-[(3-Amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-9-(1-methylaminoethyl)-7,8,9,10-tetrahydro-6,9,11-trihydroxy-4-methoxy-5,12-naphthacenedione Dihydrochloride (C-13-Methylaminohydrodaunorubicin; IV)—Methylamine hydrochloride (2.0 g, 30 mmol) and I (175 mg, 0.3 mmol) were dissolved in 20 mL of methanol. To this was added 0.3 mL of a 1.0 M methanolic solution of NaOH and, after 15 min equilibration, 1.0 mL of a freshly prepared methanolic solution of $NaCNBH_3$ (0.3 mmol/mL). The reaction was carried out at room temperature in a sealed tube for 12 h, at which time TLC of the reaction mixture on silica-gel plates (20% methanol–chloroform) indicated only the presence of IV and a trace amount of VIII. The amount of NaOH added only partially neutralizes the added amine hydrochloride. It was found experimentally that addition of larger amounts of NaOH caused the appearance of numerous unidentified side products (TLC analysis) which were difficult to remove. Addition of less NaOH caused the reaction to proceed quite slowly and allowed the formation of larger amounts of daunorubicinol, VIII. The amount of NaOH added was, thus, chosen to optimize the yield of the desired product. The methanol was evaporated under reduced pressure and then the residue was dissolved in 10 mL of pH 10.4 aqueous borate buffer (0.01 M) and extracted three times with a total of 30 mL of $CHCl_3$. The combined chloroform extracts were washed three times with distilled water, dried over Na_2SO_4, and evaporated under reduced pressure. The solid was dissolved in methanol and acidified with slightly more than two equivalents of aqueous 12 M HCl. The solvent was evaporated under reduced pressure and the residue triturated three times with 1% methanol in chloroform. At this time only IV could be detected by TLC. The dark red solid was recrystallized from ethanol–ethylacetate to give 80 mg (45%) of IV (mp, slowly dec. >179°C); TLC (silica gel, 30% CH_3OH in CH_2Cl_2): R_f 0.19; IR (KBr): 3452 (N—H), 1622, and 1589 cm^{-1} (C=O of C ring); ^{1}H-NMR (free base in $CDCl_3$): δ 8.07 (d, J = 8.0 Hz, H-1), 7.78 (t, J = 1.5 Hz, H-2), 7.37 (d, J = 8.2 Hz, H-3), 5.52 (m, H-1′), 5.28 (m, H-7), 4.06 (s, OCH_3), 4.2–3.8 (br m, OH-9, H-5′, and H-3′), 3.6–2.4 (br m, H-4′, H-10, and H-13), 2.57 (d, J = 2.8 Hz, NCH_3), 2.0–1.3 (br m, H-8 and CH_2-2′), 1.26 (d, J = 7.3 Hz, CH_3-5′), and 1.18 ppm (d, J = 6.5 Hz, CH_3-14).

Anal.—Calc. for $C_{28}H_{36}Cl_2N_2O_9$: C, 53.08; H, 6.04. Found: C, 52.90; H, 6.06.

(7*S*,9*S*)-7-[(3-Amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-9-(1-butylaminoethyl)-7,8,9,10-tetrahydro-6,9,11-trihydroxy-4-methoxy-5,12-naphthacenedione Dihydrochloride (C-13-Butylaminohydrodaunorubicin; V)—The reaction of butylamine hydrochloride with I was conducted as for IV, and the product was purified in a similar manner to yield dark red solid V in 67% yield (mp slowly dec. >160°C); TLC (silica gel, 20% CH_3OH in CH_2Cl_2): R_f 0.52; IR (KBr): 3452 (N—H), 1622, and 1581 cm^{-1} (C=O of C ring); ^{1}H-NMR (free base in $CDCl_3$): δ 8.07 (d, J = 8.0 Hz, H-1), 7.76 (t, J = 1.5 Hz, H-2), 7.39 (d, J = 8.0 Hz, H-3), 5.50 (m, H-1′), 5.26 (m, H-7), 4.08 (s, OCH_3), 4.5–3.8 (br m, OH-9, H-5′, and H-3′), 3.5–2.2 (br m, H-4′, H-10, H-13, and CH_2—α-N-butyl), 2.0–1.1 (br m, H-8, CH_2-2′, CH_2—β-N-butyl, CH_2—γ-N-butyl, and CH_2—δ-N-butyl), 1.29 (d, J = 6.4 Hz, CH_3-5′), 1.21 (d, J = 6.4 Hz, CH_3-14), and 0.94 ppm (t, J = 7.4 Hz, CH_3—N-butyl).

Anal.—Calc. for $C_{31}H_{42}Cl_2O_9N_2$: C, 55.11; H, 6.56. Found: C, 55.46; H, 6.30.

(7*S*,9*S*)-7-[(3-Amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-9-[1-(3-hydroxypranyl)aminoethyl]-7,8,9,10-tetrahydro-6,9,11-trihydroxy-4-methoxy-5,12-naphthacenedione Dihydrochloride (C-13-1-Propanolaminohydrodaunorubicin; VI)—The reaction of 3-amino-1 propanol hydrochloride with daunorubicin was conducted as for IV and the product was purified to give dark red solid VI in 38% yield (mp slowly dec. >174°C); TLC (silica gel, 20% CH_3OH in CH_2Cl_2): R_f 0.58; IR (KBr): 3443 (N—H), 1622, and 1581 cm^{-1} (C=O of C ring); ^{1}H-NMR (free base in $CDCl_3$): δ 8.05 (d, J = 7.3 Hz, H-1), 7.76 (t, J = 1.0 Hz, H-2), 7.37 (d, J = 8.2 Hz, H-3), 5.52 (m, H-1′), 5.28 (m, H-7), 4.10 (s, OCH_3), 4.5–3.6 (br m, OH-9, H-5′, H-3′), 3.5–2.2 (two m, CH_2—γ-propanol, CH_2—α-propanol, H-4′, H-10, and H-13), 1.9–1.5 (br m, H-8, CH_2-2′ and CH_2—β-propanol), 1.36 (d, J = 6.4 Hz, CH_3-5′), and 1.29 ppm (m, J = 5.5 Hz, CH_3-14).

Anal.—Calc. for $C_{30}H_{40}Cl_2N_2O_{10}$: C, 54.61; H, 5.96. Found: C, 54.71; H, 5.97.

(7*S*,9*S*)-7-[(13-Amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-9-[1-(3,3-dimethyl-1,3-diaminoethyl)]-7,8,9,10-tetrahydro-6,9,11-trihydroxy-4-methoxy-5,12-naphthacenedione Trihydrochloride (C-13-*N*,*N*-Dimethyl-1,3-propanediaminohydrodaunorubicin; VII)—The reaction of N,N-dimethyl-1,3-diaminopropane dihydrochloride with I was conducted as for IV and the product was purified to give dark red solid VII in 70% yield (slowly dec. >180°C); TLC (silica gel, 30% CH_3OH in CH_2Cl_2): R_f 0.08; IR (KBr): 3451 (N—H), 1622, and 1589 cm^{-1} (C=O of C ring); ^{1}H-NMR (free base in $CDCl_3$): δ 7.94 (d, J = 7.8 Hz, H-1), 7.72 (t, J = 1.5 Hz, H-2), 7.34 (d, J = 8.0 Hz, H-3), 5.45 (m, H-1′), 5.17 (m, H-7), 4.05 (s, OCH_3), 4.5–3.9 (br m, OH-9, H-5′, and H-3′), 3.5–2.0 (br m, H-4′, H-10, H-13, CH_2—α-N-ethylene, and CH_2—β-N-ethylene), 2.23 (s, NCH_3), 1.8–1.1 (br m, H-8, CH_2-2′), 1.29 (d, J = 7.3 Hz, CH_3-5′) and 1.09 ppm (d, J = 6.5 Hz, CH_3-14).

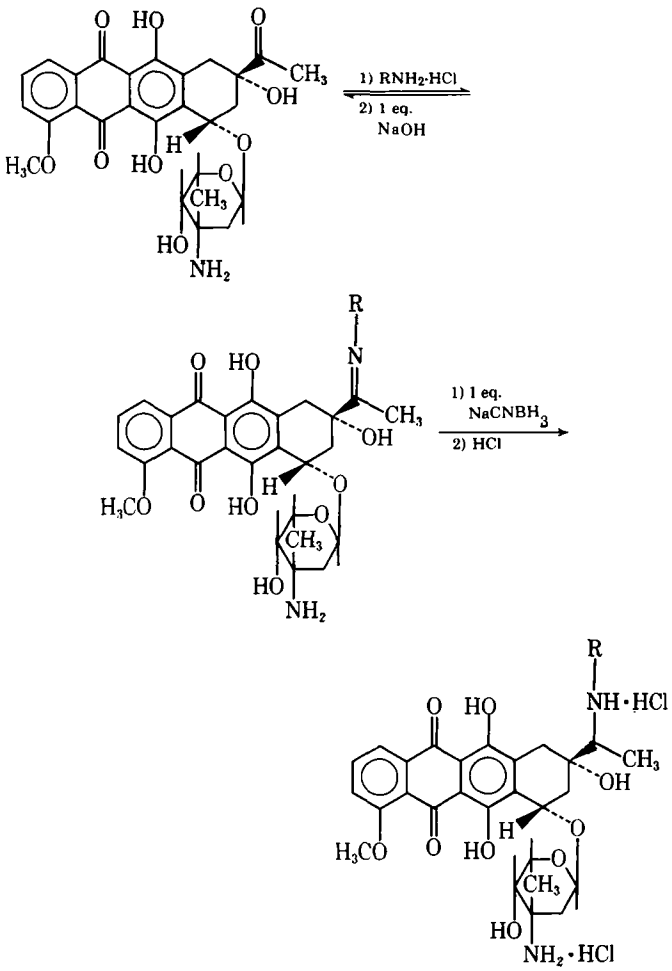
Anal.—Calc. for $C_{32}H_{46}Cl_3N_3O_9{\cdot}H_2O$: C, 51.86; H, 6.53. Found: C, 51.77; H, 6.53.

Octanol–buffer partition coefficients were determined by standard equilibration methods (15). Extinction coefficients at 480 nm, determined for the two different phases, were used for concentration determinations. Binding constants of the derivatives were determined as previously described (16) using spectrophotometric changes at 480 nm. The results were analyzed using standard methods (16–18). Viscosity measurements were made using a viscometer[2] with electronic timing as previously described (13, 14). The system for determining inhibition of *E. coli* RNA polymerase is the same as previously described for anthracycline derivatives (13). Salmon sperm DNA was used as obtained[3]. Stock solutions of DNA were made in 2 mM [1,4-piperazinebis(ethanesulfonic acid)] buffer (PIPES), pH 7.8, with NaCl added to adjust ionic strength. Anthracycline stock solutions (10 mM) were made in distilled

[1] NMR spectra were recorded from 0–10 ppm on either a JEOL GX270 or on a Varian XL-100 spectrometer. Elemental analyses were performed at Atlantic Microlab, Inc., Atlanta, Georgia. Compounds I–III were obtained from the Drug Development Branch of NCI.

[2] Beckman-Zimm rotating cylinder viscometer.

[3] Worthington Biochemical Corp.



Scheme I—Synthesis of reductive derivatives of daunorubicin, IV-VII.

water and diluted with appropriate buffer as needed.

Anthracycline-DNA complexes for biological testing were prepared by dissolving DNA (5 mM) in 0.1 mM phosphate buffer at pH 7. Anthracyclines were dissolved (0.01 M) in water and added very slowly with vigorous stirring to the DNA solution until a ratio of 1:5 of drug to DNA was obtained. The solution was warmed to 40°C with stirring until all solids had dissolved and then was lyophilized. Volumes were varied depending on the amount of drug desired.

RESULTS

Chemistry—The syntheses of IV-VII were carried out in good yield by treating daunorubicin with appropriate amines and $NaCNBH_3$ (19) as shown in Scheme I. The reactions were monitored by TLC. Typically, four spots of a reaction mixture were observed: (*a*) starting material, I; (*b*) daunorubicinol, VIII, which results from the reduction of I by $NaCNBH_3$; (*c*) a component with high mobility which is presumably the aglycon of I; (*d*) the component which after purification was identified as the desired product and which always had the smallest R_f value. Daunorubicinol, VIII, was identified by treating I with $NaCNBH_3$ directly under mild conditions. Typically, at the end of a reaction, the first three components were present in trace amount and frequently no starting material was detected.

All reactions were performed on a relatively small scale due to the expense of I. In addition to elemental analysis, the authenticity and purity of IV-VII were confirmed as follows: (*a*) NMR of aromatic, sugar, and methoxy peaks of the neutral species in $CDCl_3$ showed no significant change from I; (*b*) the C=O IR stretching vibrations of C-13 at 1712 cm^{-1} disappear after reaction; (*c*) the bands at 1622 and 1581 cm^{-1} of the two carbonyl groups of the C ring remain unchanged, indicating that the quinone is not reduced by $NaCNBH_3$; (*d*) the UV-visible spectra of the derivatives are similar to the parent compound I, indicating that the chromophore is not modified; (*e*) TLC of the purified compounds on silica gel gives only a single spot.

Biological Testing—Compounds IV-VII, along with I and II, were submitted to the National Cancer Institute for antitumor activity testing against lymphocytic leukemia P388 in mice. As shown in Table I, all derivatives are

Table I—Biological Testing Results Against Mouse Lymphocytic Leukemia [a]

Compound	NSC Number	Optimal Dose, mg/kg	Toxic Dose, mg/kg[b]	T/C[c]
I	082151	1.0	2.0	175
II	123127	1.0	3.0	204
IV	257455	8.0	16.0	159
V	263673	5.0	10.0	183
VI	266537	16.0	32.0	188
VII	256466	6.25	20.0	131

[a] All tests were arranged through the Drug Research and Development Program, Division of Cancer Treatment, National Cancer Institute. CDF mice bearing intraperitoneally implanted P388 lymphocytic leukemia (10^6 cells) on day 0 were treated on days 1-9 with the specified intraperitoneal dose of aqueous drug solutions. Detailed protocols are described in Ref. 22. The toxic dose is defined as the dose at which T/C has dropped from its maximum value to 100. [c] T/C is the mean survival time of treated/control animals, in percent, and must be ≥125 for an active result.

Table II—Binding of the Anthracycline Drugs to DNA [a]

Compound	K_0[b] × 10^{-5} (PIPES/51 mM Na^+) [c]	K_0[b] × 10^{-5} (PIPES/201 mM Na^+) [c]
I	9.3	5.8
II	14.6	10.0
IV	10.3	3.0
V	10.5	2.6
VI	5.8	1.6
VII	28.0	10.9

[a] Binding measurements were conducted using spectrophotometric changes at 480 nm as previously described (10, 11, 18). [b] K_0 represents the equilibrium constant for binding of a drug molecule to an isolated site on the DNA double helix. It is determined from the intercept on the vertical axis of a Scatchard plot of binding data. [c] 2 mM PIPES buffer, pH 7.8, with 40 mM NaCl added. [d] 2 mM PIPES buffer, pH 7.8, with 200 mM NaCl added.

active with V and VI showing greater activity than the parent compound I. Relative to I and II, all derivatives require larger amounts of compound (mg/kg) to reach both the optimum and toxic doses (Table I). The ratio of toxic to the optimal dose is approximately two for I and is not significantly increased for any of the derivatives. The activity of V and VI is quite encouraging and we have, therefore, analyzed the interaction of all of these compounds with DNA and have measured their octanol-water partition coefficients to determine how the biological testing results correlate with the binding and the partitioning properties.

Mode of Binding—Since I and II are known to bind to DNA by intercalation, IV through VII might also be expected to intercalate. A consistent indicator of intercalation is the ability of a drug to increase the length of linear DNA (20). All of the derivatives gave large increases in viscosity, as expected for intercalators in the presence of linear DNA[4] (16). The increases, at similar amounts of bound drug, were only ~80% of that obtained with I and II, indicating that replacing the keto function by the amino substituents perturbs the interaction of the anthracycline ring system with the double helix. It seems conclusive that all of the derivatives bind to DNA by intercalation, but the structure of the complex formed could be somewhat different from that for I and II with DNA.

DNA-Induced Absorption Spectral Changes and DNA Binding Studies—The anthracycline chromophore has an intense visible absorption band near 480 nm which exhibits shifts to longer wavelengths and hypochromism on intercalation with DNA. Similar effects are seen for the derivatives IV-VII as for I and II. These spectral changes can be used to calculate equilibrium constants for the interaction of the anthracyclines with DNA (14, 16). Because the binding of these compounds to DNA is complex (17), their interactions with DNA have been compared by determining the intercept on the vertical axis from Scatchard plots of spectrophotometric binding data. This limit should be the apparent equilibrium constant, K_0, for binding of a drug molecule to an isolated binding site in DNA (16). This method has the advantage of giving accurate relative binding strengths for a series of derivatives while not requiring any specific assumption about binding models for the DNA-drug interaction. A similar treatment has been used to compare the binding of antitumor platinum drugs with DNA (18). The results for derivatives IV-VII compared to I are shown in Table II at two different ionic strengths.

Inhibition of *Escherichia coli* RNA Polymerase—If the anthracyclines function in part by binding to DNA, they should exert dramatic effects on the biological as well as the physical properties of DNA. The potential biological inhibition in a standard *E. coli* RNA polymerase assay system (9) was mon-

[4] Unpublished results.

Table III—Relative Inhibition of DNA-Dependent *E. coli* RNA Polymerase[a]

Compound	Enzymatic Activity, %
none (control)	100
I	9
II	9
IV	10
V	13
VI	10
VII	5

[a] The kinetics of incorporation of [^{3}H]UMP into RNA using *E. coli* RNA polymerase was monitored for the compounds indicated. Heterogeneous calf thymus DNA (0.12 mM P/L) was used as the template for nonspecific RNA polymerization. Drugs were added at 0.12 mM concentration and reductions in activity of RNA polymerase monitored as previously described (13).

Table IV—1-Octanol–Buffer Partition Coefficients

Compound	Partition Coefficient[a]
I	2.8
II	0.5
IV	0.3
V	4.3
VI	0.8
VII	0.1

[a] Determined spectrophotometrically between 1-octanol saturated with buffer (2 mM PIPES, pH 7.8, with 20 mM NaCl) and octanol saturated buffer using standard procedures (21).

Table V—Biological Activity of Anthracycline–DNA Complexes[a]

Compound[b]	NSC Number	Optimal Dose, mg/kg[c]	Toxic Dose, mg/kg	T/C, %
I	082151	1.0	2.0	175
I-nDNA	257454	0.83	5.2	217
I-sDNA	257453	1.67	4.5	197
I-dDNA	262198	0.83	3.2	182
V-nDNA	263674	6.7	>50[d]	224
VI-nDNA	266538	>8[d]	>8[d]	173
VII-nDNA	262650	6.7	13	145

[a] Testing results and column headings are as in Table I. [b] All compounds were prepared at a 1:5 ratio of drug to DNA as described in the *Experimental Section*: nDNA is native salmon sperm DNA; sDNA is sonicated; dDNA is denatured. [c] Doses are calculated based on the amount of drug in the DNA–drug complex. The actual dose of the complex was six times greater than that indicated. [d] This indicates the maximum dose administered in the test. The optimum or toxic doses may be greater than this value.

itored and the results are shown in Table III. As with other anthracycline derivatives, the polymerase inhibition results parallel binding interactions (13, 14) and strong inhibition of the polymerase is obtained for IV–VII.

Partition Coefficients—The charged amine function on an already slightly polar parent molecule obviously has the potential for reducing membrane permeability. Octanol–buffer partition coefficients (15) were determined to assess the relative ability of the derivatives to pass through membranes as compared to I and II. The partition coefficients are listed in Table IV and, as expected, show decreases for IV, VI, and a large decrease for VII. The butyl derivative actually partitions better into the organic phase than I.

DNA–Anthracycline Complexes: Biological Testing—It has been proposed that complexes of drugs such as the anthracyclines with suitable biopolymers should be absorbed faster into tumor cells than into normal cells due to the higher potential endocytic activity of tumor cells (21). Since the absorption of drugs through their macromolecular complexes should depend much less on their partition coefficients than absorption of free drugs, complexes of I, II, V, VI, and VII with DNA were prepared. The testing results for these complexes are shown in Table V. As can be seen from the results with I, the physical state of the DNA has some effect on activity, with the native high molecular weight DNA complex giving higher activity than the sonicated or denatured samples. The complex of I with native DNA has higher activity than I alone, has a similar optimum dose (based on the amount of drug in the dose), and has a significantly improved chemotherapeutic index as compared with uncomplexed I. The results with the derivatives indicate similar behavior. The complex with VI could not be thoroughly investigated due to limitations in the amount of material available. The complex with VII was better than the free drug but not as significantly improved as one might expect based on its DNA binding properties. The complex with V shows marked improvement and has the highest activity in this series.

DISCUSSION

All of the derivatives of this study have been shown to bind strongly to DNA through an intercalation complex. The enhanced binding of IV and V relative to I is due to increased sodium ion release by these compounds on complexation with DNA (16). This might also partially account for the greater binding of VII, but the large enhancement for this compound, even at higher ionic strength, indicates some additional favorable interactions with DNA. At the higher ionic strength, which is closer to physiological conditions, only VII binds to DNA better than I. The slightly reduced binding of VI could be due to problems of hydration of the alcohol in the complex *versus* the free solution state.

Compounds IV and V bind quite similarly to DNA (Table II) whereas VII binds much more strongly to DNA than IV or V but partitions poorly (Table IV) and has the lowest activity of all of the compounds of this series. The propanolamine derivative VI has a lower binding constant than the other analogues (although it still binds strongly to DNA), and it has an intermediate partition coefficient. This compound has the highest *in vivo* activity of any of the derivatives and is more active than the parent compound I. Although there are too few compounds here to construct a structure–activity plot, it is interesting to note that dauxorubicin hydrochloride also has high activity coupled with a high DNA binding constant and an intermediate partition coefficient. This suggests that activity for those anthracycline derivatives which bind strongly to DNA and do not have other complicating factors might be found to peak at a partition coefficient of ~0.7. The low dose of II required relative to VI could be due, in part, to the larger DNA binding constant of II (Table II). Further investigation with a series of anthracyclines with partition coefficients of ~0.7 and high DNA binding constants would be of interest.

Because of the high DNA binding constant and low partition coefficient for VII it was expected that a dramatic enhancement in activity against P388 might be obtained for this compound through preparation and administration of a DNA complex. This idea is based on the so called "lysosomotropic" principle, which predicts selective uptake by antineoplastic cells of high molecular weight macromolecule–drug complexes. This delivery system could potentially increase the efficacy of the complexed drug relative to the free species, especially for derivatives such as VII which have low partition coefficients but bind strongly to DNA. As can be seen by comparison of biological testing results on VII in Tables I and V, although activity was increased, the results were not remarkable. All of the compounds analyzed as DNA complexes had similar optimal doses both complexed and uncomplexed. The toxic doses were generally significantly increased in the complexes relative to the uncomplexed derivatives. This suggests that the DNA is acting more to prevent toxicity than enhance activity. Obviously, if toxicity is lowered, the mean survival time for treated/control animals (T/C) at the maximum could rise slightly as the probability of a toxic reaction is shifted to higher dose levels relative to the optimum dose. Metabolic reactions, such as aglycon formation, would also be expected to be slower when an anthracycline is bound to DNA; this may be one of the major advantages of administering these compounds as DNA complexes. The activity of the V–DNA complex is encouraging and this compound and its DNA complex are being further investigated in *in vivo* systems.

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Liquid Membrane Phenomenon in Diuretics

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Abstract □ Surface activity and critical micelle concentrations are reported for two diuretic drugs, furosemide and triamterene. The drugs generate a liquid membrane on a supporting membrane. Transport of chloride, sodium, and potassium ions through the liquid membranes generated by the drugs was studied. The data suggest that the phenomenon of liquid membrane formation may contribute to the diuretic action.

Keyphrases □ Diuretics—liquid membrane phenomenon, critical micelle concentrations, chloride, sodium, potassium ion transport, furosemide, triamterene □ Furosemide—liquid membrane phenomenon, anion/cation transport □ Triamterene—liquid membrane phenomenon, anion/cation transport

Drugs known to act by altering the permeability of cell membranes are mostly surface active. Surface-active agents added to an aqueous phase are known to generate a surfactant-layer liquid membrane at the interface which modifies material transport across it (1). It is therefore likely that the liquid membranes generated by surface-active drugs play a role in the mechanism of drug action. A wide category of surface-active drugs have been investigated in this laboratory (2-7), and results indicated that the liquid membranes contribute to the mechanism of drug action.

Most of the high-ceiling diuretics (8) are known to act by altering the reabsorption of cations (*e.g.*, Na^+) and anions (*e.g.*, Cl^-) in the ascending limb of the loop of Henle (8). Although diuretics act by modifying the membrane permeability, their surface activity is not documented in the literature. In the present study, surface activity of these drugs is demonstrated—critical micelle concentrations (CMC) have been determined—and the existence of liquid membranes generated by them at the interface is confirmed. Transport of relevant cations and anions in the presence of the liquid membranes generated by the drugs has been studied. The data indicate that the liquid membranes generated by the diuretic drugs contribute to the mechanism of their action. A cellulose nitrate microfiltration membrane/aqueous interface was chosen as a site for the formation of the liquid membranes to eliminate the possibility of active and specific interaction of the drugs with the constituents of the biological membranes and to highlight the role of passive transport through the liquid membranes. Two structurally dissimilar diuretic drugs (furosemide and triamterene) were chosen for the present study.

EXPERIMENTAL SECTION

Material—Furosemide[1], triamterene[2], sodium chloride[3], potassium chloride[3], and water (glass distilled over potassium permanganate) were used.

Methods—The critical micelle concentration (CMC) of aqueous furosemide and triamterene solutions were determined from the variation in surface tension with concentration at 37 ± 0.1°C. The surface tensions were measured using a tensiometer[4]. To prepare an aqueous solution of furosemide, the drug

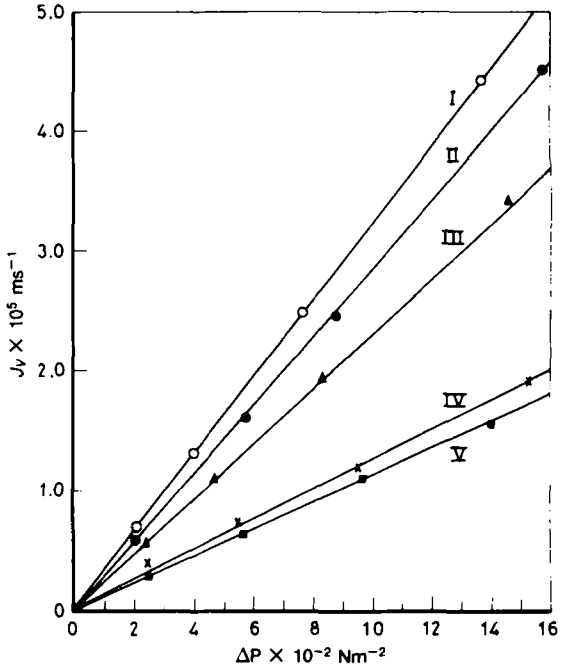

Figure 1—*Hydraulic permeability data. Curves I, II, III, IV, and V are for 0, 2.08 × 10^{-5}, 4.16 × 10^{-5}, 8.3 × 10^{-5}, and 24.9 × 10^{-5} M furosemide concentrations, respectively.*

[1] Hoechst Pharmaceutical, Ltd.
[2] S, K & F Ltd.
[3] Analytical grade.
[4] Model 21 Tensiomat; Fisher.