Adriamycin Analogues. 3. Synthesis of N-Alkylated Anthracyclines with Enhanced Efficacy and Reduced Cardiotoxicity

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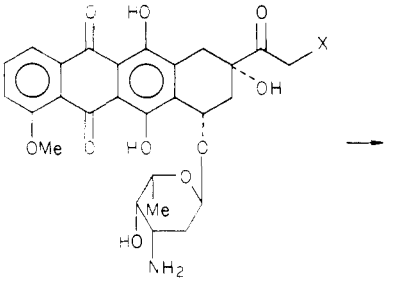
Reaction of daunorubicin (1) and adriamycin (2) with aldehydes and ketones in the presence of $NaCNBH_3$ afforded *N*-alkyl- and *N,N*-dialkylanthracyclines along with their 13-dihydro derivatives. Product ratios depended upon the nature of the carbonyl reagent and the starting drug. The majority of these analogues retained in vivo antitumor activity comparable to 1 and 2. However, unlike the parent compounds, which inhibit DNA and RNA synthesis at comparable concentrations, several of these analogues inhibit RNA synthesis at markedly lower concentrations than required to inhibit DNA synthesis. In addition, in some cases the ability to bind to DNA in vitro was reduced while antitumor activity was retained. *N,N*-Dibenzyldaunorubicin was especially notable for increased efficacy (T/C 259, qd 1–9) against P388 leukemia in mice, despite reduction of DNA binding in vitro. It showed almost complete loss of mutagenicity vs. *S. typhimurium* (Ames test) and it was tenfold less cardiotoxic by electrocardiographic measurements (Zbinden test) in the rat.

The anthracycline antibiotics daunorubicin[2] (1) and adriamycin[3] (2) are clinically useful antineoplastic agents, with adriamycin having an especially broad spectrum of activity, extending to certain solid tumors that are normally resistant to most modes of chemotherapy. However, the clinical use of these drugs is hampered by a number of undesirable side effects, the most serious being a dose-related and irreversible cardiotoxicity.[4] As part of our efforts to prepare analogues of 1 and 2 having improved therapeutic properties, we now report the synthesis and biological evaluation of a number of *N*-alkyl and *N,N*-dialkyl derivatives of 1 and 2.

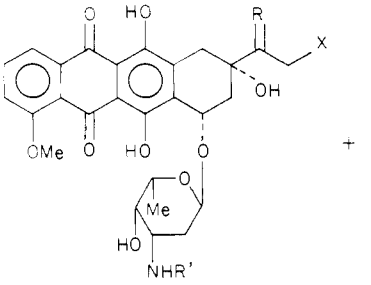
The antitumor activity of 1 and 2 is thought to be dependent in part on their ability to bind to nuclear DNA via an intercalative mechanism.[3d] In this process, the protonated 3′-amino function is critical as it stabilizes the drug–DNA complex via formation of an electrostatic bond with the DNA phosphate. Therefore, one would expect modifications at this position to have a marked effect on the biological activity. For example, Zunino et al. reported that 3′-epi analogues of daunorubicin bind to DNA less strongly than do compounds having the natural configuration at C-3′.[5] Likewise, *N*-acylation also inhibits the binding of drug to DNA and consequently reduces both the efficacy and potency in most cases.[6] This is presumably due to the loss of basicity upon acylation, preventing the formation of the electrostatic bond with the DNA phosphate. However, upon alkylation the basicity of the 3′-amino function would be retained, making it possible to prepare analogues which could bind to DNA yet have different lipophilic properties and varying degrees of steric bulk about the 3′-amino group.

Such analogues have now been prepared. Among these, *N,N*-dibenzyldaunorubicin (**5g**, X = H) proved to be of special interest. In spite of increased lipophilic character and steric bulk about the amino nitrogen, **5g** (X = H) displayed enhanced antitumor activity even though the ability to bind to DNA is lost. The cardiotoxic and mutagenic side effects of the parent compounds appear to be greatly reduced in the dibenzyl derivative as well.

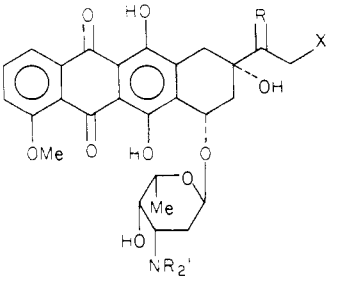
Chemistry. The reductive alkylation procedure developed by Borch et al.[7] afforded ready access to this class of compounds. Reaction of the parent drug in aqueous acetonitrile with $NaCNBH_3$ and the appropriate carbonyl reagent at ambient temperature afforded the *N*-alkyl and *N,N*-dialkyl compounds, along with their 13-dihydro derivatives, in varying ratios depending on the nature of the carbonyl reagent and the starting drug. In general, smaller aldehydes, such as formaldehyde and acetaldehyde, gave only dialkyl derivatives, while more sterically hindered reagents, such as acetone and pivalaldehyde, afforded exclusively monoalkyl derivatives, despite use of the reagent in large excess. In other cases, such as with benzaldehyde or decylaldehyde, the ratio of mono- and dialkylated products was dependent on the reaction time, and either product could be prepared selectively. For example, reaction of 1 with excess benzaldehyde afforded



1, X = H
2, X = OH



3, R = O
4, R = OH, H



5, R = O
6, R = OH, H

a, R′ = CH_3; b, R′ = C_2H_5; c, R′ = $CH(CH_3)_2$;
d, R′ = $(CH_2)_5$; e, R′ = $(CH_2)_8CH_3$; f, R′ = $CH_2C(CH_3)_3$;
g, R′ = $CH_2C_6H_5$; h, R′ = $CH_2C_6H_4$-*p*-NMe_2

after 30 min predominantly the monoalkyl product **3g** (X = H), while after 7 days the dialkylated product **5g** (X = H) was obtained almost exclusively. An apparently

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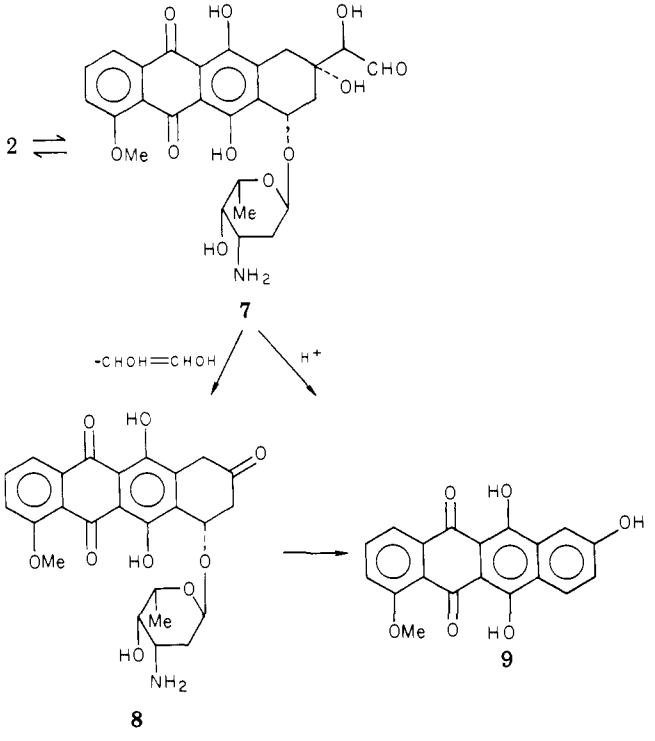
Table I. Reaction Conditions and Product Distribution for the Reductive N-Alkylations of Daunorubicin and Adriamycin in This Study

substrate	reagent	reaction time, equiv of carbonyl reagent	R'	X	products (% yield)[a] 3	4	5	6
1	CH_2O	30 min, 10	Me	H			a (80)	a (6)
2	CH_2O	30 min, 10	Me	OH			a (43)	
1	MeCHO	20 h, 20	Et	H			b (37)	
2	MeCHO	16 h, 30	Et	OH			b (22)	
1	Me_2CO	16 h, 20	*i*-Pr	H	c (42)[b]			
1	$OHC(CH_2)_3CHO$	4 h, 1	$(CH_2)_5$	H			d (30)[c]	d (11)[c]
2	$OHC(CH_2)_3CHO$	2 h, 2	$(CH_2)_5$	OH			d (11)	d (19)
1	$Me(CH_2)_8CHO$	2 h, 20	$Me(CH_2)_9$	H	e (37)			
1	$Me(CH_2)_8CHO$	3 h, 40	$Me(CH_2)_9$	H	e (3)		e (46)	e (4)
1	Me_3CCHO	2 h, 20	Me_3CCH_2	H	f (67)	f (10)[c]		
1	C_6H_4CHO	30 min, 20	Bzl	H	g (49)	g (6)	g (5)	
1	C_6H_5CHO	7 days, 20	Bzl	H	g (1)		g (70)	c (4)
2	C_6H_5CHO	30 min, 20	Bzl	OH	g (30)	g (38)	g (3)	
1	p-$Me_2NC_6H_4CHO$	4 days, 20	p-$Me_2NC_6H_4CH_2$	H	h (60)	h (18)		

[a] Yields refer to isolated products, homogenous by TLC, and displaying the expected UV, IR, and NMR spectra. Acceptable C, H, N analyses were obtained for all new compounds except where noted. [b] Anal. ($C_{37}H_{49}NO_{10}\cdot HCl\cdot H_2O$) C, N, Cl; H: calcd 6.14; found, 5.46. [c] Isolated and characterized as the free base.

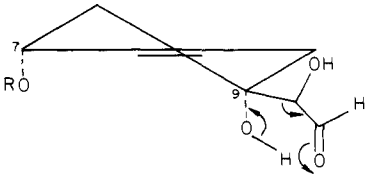
Scheme I



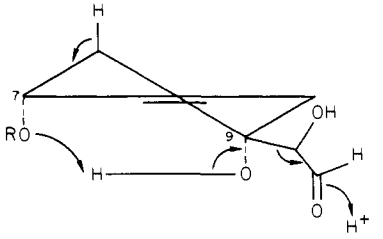
anomalous result was obtained with *p*-(dimethylamino)-benzaldehyde. Even after extended reaction periods, the monoalkyl compound **3h** (X = H) was the predominant product. As steric factors would not be expected to be significantly different than in the benzaldehyde case, a possible explanation may be a lowering of the electrophilicity of the aldehyde carbonyl due to the electron-donating resonance effect of the *p*-(dimethylamino) group. Further work, however, is needed to clarify this point.

The only significant side reaction observed is reduction of the 13-carbonyl, affording N-alkylated 13-dihydro derivatives, which were readily identified by the disappearance of the 13-carbonyl absorbance in the IR spectra. This reduction was especially apparent when adriamycin served as the substrate. The products were separated by silica gel chromatography and converted to the HCl salts for isolation. The yields and reaction conditions employed to obtain the compounds prepared in this study are outlined in Table I. Table II outlines the NMR data characteristic of each compound. The analytically pure HCl salts were submitted as amorphous powders for biological evaluation.

Mass recoveries in the adriamycin series are generally lower than in the corresponding daunorubicin reactions. The greater instability of adriamycin and its derivatives probably accounts for this, and in support of this hypothesis we have isolated naphthacenedione **9**. This material was apparently generated during chromatography, as it was undetectable in the crude reaction mixture and appeared to bleed steadily off the column in an approximate yield of 5%. This exceedingly mild 2-carbon degradation may be explained by tautomerization of the α-hydroxy ketone side chain of **2** to the α-hydroxy aldehyde **7** (Scheme I). Degradation of **7** to the 9-ketone **8** may be envisioned via a retro-aldol process, possibly proceeding through a six-membered transition state such as A, followed by β-elimination of the duanosamine moiety



A



B

and tautomerization to **9**. Alternatively, the retro-aldol, β-elimination sequence may be considered as an acid-catalyzed concerted process, as illustrated in mechanism B. Penco et al. have recently reported the conversion of **8** to **9**.[8]

Results and Discussion

The biological data for the N-alkylated anthracyclines prepared in this study are presented in Table III. In general, the N-alkylated anthracyclines with small R groups (**a–d**) are qualitatively similar to the parent drugs.

Table II. ^{1}H NMR Data for N-Alkylated Anthracyclines[a]

compound[d]	solvent[b]	chemical shift data, δ (multiplicity, number of protons)	
		14-H	N substituent
N,N-Me_2dnr (5a, X = H)	Me_2SO-d_6	2.32 (s, 3)	2.76 (s, 6, NMe_2)
N,N-Me_2-13-dihydrodnr (6a, X = H)	$CDCl_3$	1.28 (d, 3)	2.22 (s, 6, NMe_2)
N,N-Me_2adm (5a, X = OH)	Me_2SO-d_6	4.64 (s, 2)	2.76 (s, 6, NMe_2)
N,N-Et_2dnr (5b, X = H)	Me_2SO-d_6	2.34 (s, 3)	1.18 (m, 6, NCH_2CH_3), 3.25 (m, 4, NCH_2CH_3)
N,N-Et_2adm (5b, X = OH)	Me_2SO-d_6	4.67 (s, 2)	1.18 (m, 6, NCH_2CH_3), 3.22 (m, 4, NCH_2CH_3)
N-*i*-Prdnr (3c, X = H)	Me_2SO-d_6	2.33 (s, 3)	1.23 [d, 6, $CH(CH_3)_2$], 3.44 [m, 1, $CH(CH_3)_2$]
N,N-pentamethylenednr (5d, X = H)	$CDCl_3$	2.43 (s, 3)	1.50 [m, 6, $NCH_2(CH_2)_3CH_2$], 2.50 (m, 4, NCH_2)
N,N-pentamethyleneadm (5d, X = OH)	$CDCl_3$	4.80 (s, 2)	1.50 [m, 6, $NCH_2(CH_2)_3CH_2$], 2.50 (m, 4, NCH_2)
N,N-pentamethylene-13-dihydroadm (6d, X = OH)	$CDCl_3$	2.80 (m, 2)	1.60 [m, 6, $NCH_2(CH_2)_3CH_2$], 2.50 (m, 4, NCH_2)
N-decyldnr (3e, X = H)	$CDCl_3$	2.43 (s, 3)	0.86 [m, 3, $N(CH_2)_9Me$], 1.0-1.6[c] [m, 16, $NCH_2(CH_2)_8Me$], 1.9–2.6[c] (m, 2, NCH_2)
N-decyl-13-dihydrodnr (4e, X = H)	$CDCl_3$	1.0-1.6[c] (m, 3)	0.84 [m, 3, $N(CH_2)_9Me$], 1.0–1.6[c] [m, 16, $NCH_2(CH_2)_8Me$], 2.0–2.6[c] (m, 2, NCH_2)
N,N-didecyldnr (5e, X = H)	$CDCl_3$	2.43 (s, 3)	0.90 [m, 6, $N(CH_2)_9Me$], 1.0–1.6[c] [m, 32, $NCH_2(CH_2)_8Me$], 2.0-2.8[c] (m, 4, NCH_2)
N,N-didecyl-13-dihydrodnr (6e, X = H)	$CDCl_3$	1.0–1.6[c] (m, 3)	0.86 [m, 6, $N(CH_2)_9Me$], 1.0–1.6[c] [m, 32, $NCH_2(CH_2)_8Me$], 2.2–3.6[c] (m, 4, NCH_2)
N-decyladm (3e, X = OH)	$CDCl_3$	4.80 (s, 2)	0.6–0.9 [m, 3, $N(CH_2)_9Me$], 1.0–1.5[c] [m, 16, $NCH_2(CH_2)_8Me$], 1.9-3.5[c] (m, 2, NCH_2)
N-pivalyldnr (3f, X = H)	$CDCl_3$	2.43 (s, 3)	0.94 [s, 9, $C(Me)_3$], 2.36 (s, 2, NCH_2)
N-pivalyl-13-dihydrodnr (4f, X = H)	$CDCl_3$	1.35 (d, 3)	0.94 [s, 9, $C(Me)_3$], 2.36 (s, 2, NCH_2)
N-Bzldnr (3g, X = H)	$CDCl_3$	2.43 (s, 3)	3.77 (d, 2, NCH_2), 7.38 (s, 5, CH_2Ar)
N-Bzl-13-dihydrodnr (4g, X = H)	$CDCl_3$	1.35 (d, 3)	3.75 (d, 2, NCH_2), 7.38 (s, 5, CH_2Ar)
N,N-Bzl_2dnr (5g, X = H)	$CDCl_3$	2.43 (s, 3)	3.75 (s, 4, NCH_2), 7.35 (s, 10, CH_2Ar)
N,N-Bzl_2-13-dihydrodnr (6g, X = H)	$CDCl_3$	1.35 (d, 3)	3.75 (s, 4, NCH_2), 7.24 (s, 10, CH_2Ar)
N-Bzladm (3g, X = OH)	$CDCl_3$	4.78 (s, 2)	3.77 (d, 2, NCH_2), 7.30 (s, 5, CH_2Ar)
N-Bzl-13-dihydroadm (4g, X = OH)	$CDCl_3$	2.78 (d, 2)	3.77 (d, 2, NCH_2), 7.30 (s, 5, CH_2Ar)
N,N-Bzl_2adm (5g, X = OH)	$CDCl_3$	4.74 (s, 2)	3.77 (s, 4, NCH_2), 7.30 (s, 10, CH_2Ar)
N-*p*-NMe_2Bzldnr (3h, X = H)	$CDCl_3$	2.43 (s, 3)	2.89 (s, 6, NMe_2), 3.71 (br s, 2, CH_2Ar), 6.61 (d, 2, *o*-Ar H's), 7.14 (d, 2, *m*-Ar H's)
N-*p*-NMe_2Bzl-13-dihydrodnr (4h, X = H)	$CDCl_3$	1.30 (m, 3)	2.89 (s, 6, NMe_2), 3.66 (br s, 2, CH_2Ar), 6.64 (d, 2, *o*-Ar H's), 7.10 (d, 2, *m*-Ar H's)

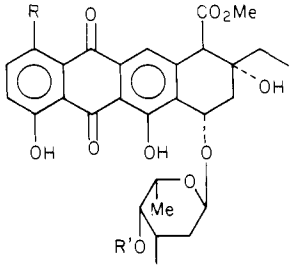
[a] Chemical shifts for protons other than those specified approximate the data reported under the Experimental Section. [b] Spectra reported in Me_2SO-d_6 are of the HCl salts, while those reported in $CDCl_3$ are of the free bases. [c] Approximate value, signal obscured by overlapping peaks. [d] Abbreviations used: dnr, daunorubicin; adm, adriamycin.

Table III. Comparison of Biological Data for Daunorubicin, Adriamycin, and N-Alkyl Analogues

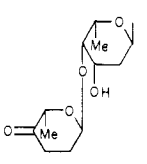
compound	isolated helical DNA ΔT_m, °C[a]	L-1210 cells: nucleic acid synth inhibn,[a] ED_{50}, μM DNA	RNA	ED_{50} ratio DNA/RNA	antitumor activity in mice[b] P388, qd 1-9 opt dose, mg/kg	T/C[c] (n)[d]	P388, q4d 5, 9, 13 opt dose, mg/kg	T/C (n)
daunorubicin (1)	11.2	0.66	0.33	2.0	0.78	160 ± 27 (8)	8	132 ± 4 (29)
adriamycin (2)	13.4	1.5	0.58	2.6	0.78	197 ± 26 (8)	8	159 ± 20 (29)
N,N-Me_2dnr (5a, X = H)	15.3	0.67	0.064	10.4	2.0	214 ± 14 (2)	10	145
N,N-Me_2adm (5a, X = OH)	17.5	0.62	0.12	5.2	0.5	164	6	125
N,N-Et_2dnr (5b, X = H)	13.6	0.67	0.13	5.2	4.0	178 ± 4 (2)		
N,N-Et_2adm (5b, X = OH)	14.6	0.70	0.21	3.3	3.13	171 ± 6 (2)		
N-*i*-Prdnr (3c, X = H)	10.6	1.1	0.32	3.4	12.5	199		
N-$(CH_2)_5$adm (5d, X = OH)	15.4	0.70	0.04	17.5	3.13	190	9.4	155
N-$(CH_2)_3$-13-dihydroadm (6d, X = OH)	12.5	0.72	0.10	7.2	1.56	157	18	143 ± 9 (2)
N-decyldnr (3e, X = H)	7.0	3.3	2.6	1.3		<125		
N,N-didecyldnr (5e, X = H)	0.1	280	130	2.1		<125		
N-Bzldnr (3g, X = H)	10.2	1.6	0.17	9.4	3.12	185	18.8	184 (3)
N-Bzl-13-dihydrodnr (4g, X = H)	6.25	1.7	0.32	5.3	6.25	221 ± 5 (2)		
N,N-Bzl_2dnr (5g, X = H)	1.35	>100	10	>10	25.0	259 ± 12 (2)	37.5	209 ± 17 (2)
N,N-Bzl_2-13-dihydrodnr (6g, X = H)	0.6	220	7.6	29.1	12.5	231 ± 2 (2)	25	183 ± 11 (2)
N-Bzladm (3g, X = OH)	11.3	0.65	0.09	7.2	3.13	185 ± 10	18.8	190 ± 10 (2)
N-Bzl-13-dihydroadm (4g, X = OH)	7.6	1.4	0.29	4.8	1.56	176 ± 20 (2)	37.5	135 ± 8 (2)
N,N-Bzl_2adm (5g, X = OH)	0	110	4.8	22.8	6.25	175		

[a] ΔT_m and ED_{50} values were determined by the method of G. Tong, W. W. Lee, D. R. Black, and D. W. Henry, *J. Med. Chem.*, 19, 395 (1976), except that the drugs were initially dissolved in a volume of Me_2SO that resulted in a final Me_2SO concentration of 5 and 1%, respectively, in the assay medium. This modification greatly aided solubilization and did not affect assay results, according to extensive control experiments. ΔT_m values < 1 indicate an insignificant degree of binding to DNA. [b] Assays arranged through Drug Research and Development Program, Division of Cancer Treatment, National Center Institute. BDF or CDF mice were injected ip with 10^6 P388 lymphocytic leukemia cells on day 0 and were treated ip on days 1-9 or days 5, 9, 10 with the specified drug dose. For detailed protocols, see R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, 3 (2), 9 (1972). [c] Ratio of average survival times of treated mice to untreated controls in percent. The average survival time of untreated controls is approximately 11 days. Activity is defined as values of T/C ≥ 125 in the qd 1-9 protocol and T/C ≥ 120 in the q4d 5, 9, 13 protocol. [d] *n* = number of tests, if more than 1.

These compounds bind strongly to DNA as indicated by their ΔT_m value, which is a measure of the stabilization of helical DNA to thermal denaturation imparted by the drug. They also display in vivo activity at low doses in the P388 mouse leukemia tumor screens comparable to **1** and **2**. However, while the parent compounds inhibit in vitro DNA and RNA synthesis at approximately the same levels, several of the N-alkylated derivatives inhibit RNA synthesis at markedly lower concentrations than those required to inhibit DNA synthesis, as indicated by the high ratio of 50% inhibitor doses (ED_{50}) for DNA/RNA. Similarly high DNA/RNA ratios have been reported for aclacinomycin A (**10**) and a number of related anthracyclines having an amino di- or trisaccharide residue at C-7.[9] This has been proposed[9] as the basis for the existence of another class of anthracyclines (class II) that are selectively inhibitory toward RNA synthesis and which appear to show improved biological properties. For example, besides possessing potent antitumor activity, **10** has been shown to be substantially less cardiotoxic than adriamycin, as determined by ECG monitoring of hamsters.[10] Cinerubin A (**11**), another compound of this type, possesses low but reproducible activity against an adriamycin-resistant subline of P388 leukemia.[11]



10, R = H; R′ =
11, R = OH; R′ =



12, R = R′ = H

In the *N*-alkylanthracycline series the trend of strong binding to DNA and potent in vivo activity continues with the monobenzyl derivatives. However, the dibenzyl derivatives, such as **5g** (X = H), exhibit markedly different properties. They show reduced binding to DNA, as judged by their ΔT_m values, and are less potent inhibitors of in vitro nucleic acid synthesis while still showing selectivity against RNA synthesis. However, they are highly efficacious in vivo, surpassing the parent compounds albeit at higher doses.

Data from cardiotoxicity tests in the Zbinden rat model[12] (Table IV) are especially striking. The dimethyl analogues show no advantage over the parent compounds, with dimethyladriamycin (**5a**, X = OH) appearing to be twice as toxic[13] in the Zbinden assay. On the other hand, the minimum total dose of dibenzyldaunorubicin (**5g**, X = H) leading to significant alterations in the ECG is more than ten times that of adriamycin. This tenfold decrease in cardiotoxicity observed for **5g** (X = H) is greater than that for any other active compound in this assay. The predictive value of these data for human therapy has yet to be established, but the Zbinden assay is the best short-term test for anthracycline cardiotoxicity currently available[14] and, thus, indicates considerable interest in **5g** (X = H) in spite of its reduced potency relative to the parent compounds against P388 leukemia in the mouse. Long-term tests in other species are in progress at the National Cancer Institute.

Table IV. Cardiotoxicity and Mutagenicity Data for Daunorubicin, Adriamycin, and Selected N-Alkyl Analogues

compound	cardiotoxicity:[a] min cum cardiotoxic dose, mg/kg	mutagenicity,[b] revertants produced/nmol of compd	dose range, μg/plate	toxicity level,[c] μg/plate
daunorubicin (**1**)	14	100 ± 30	0-20	5-12
adriamycin (2)	11			
N,N-Me_2dnr (5a, X = H)	11-16	0	0-50	20
N,N-Me_2adm (5a, X = OH)	6	1	0-50	20
N,N-pentamethyleneadm (5d, X = OH)		1	0-50	20
N-Bzldnr (**3g**, X = H)		0.5	0-20	
N,N-Bzl_2dnr (5g, X = H)	125	0.5	0-100	
N,N-Bzl_2-13-dihydrodnr (6g, X = H)		0.5	0-20	

[a] Cardiotoxicity in rats; assay described in ref 8. [b] Tests were run as described by J. McCann, E. Choi, E. Yamasaki, and B. N. Ames, *Proc. Natl. Acad. Sci. U.S.A.*, 72 5135 (1975), on *Salmonella typhimurium* tester strain TA98 [J. McCann, N. E. Spingarn, J. Kobori, and B. N. Ames, *Proc. Natl. Acad. Sci. U.S.A.*, 72, 979 (1975)]. Values for daunorubicin are from nine experiments. In the compounds reported here, no significant difference was observed between results obtained with and without microsomal activation. [c] Level at which test compound begins to kill bacteria. Mutagenicity calculated from data up to this point.

Another striking contrast is the almost complete loss of mutagenicity for the *N*-alkylated anthracyclines in the Ames test as compared with the parent compounds. Daunorubicin and adriamycin are among the most potent mutagens tested in *Salmonella* strains[15] and have also been shown to induce tumors in rats at low doses.[16] These findings are related to other observations about DNA as a receptor site for the anthracyclines (vide supra), but they also mean that **1** and **2** are at least suspect as human carcinogens.[17] While this is a minor concern compared with the more immediate side effects, it must be kept in mind in cases of long-term survival following anthracycline therapy. The binding properties of *N,N*-dibenzyldaunorubicin raise the possibility that it may also differ from the parent compounds in its mutagenic/carcinogenic potential. Supporting this hypothesis is the almost complete loss of mutagenicity in going from **1** to **5g** (X = H) in the Ames test, even after microsomal activation. *N*-Benzyldaunorubicin (**3g**) and *N,N*-dibenzyl-13-dihydrodaunorubicin (**6g**), potential in vivo metabolites of **5g** (X = H), also show nonmutagenicity by this procedure.

The loss of mutagenicity by the *N,N*-dimethyl and piperidino analogues is harder to rationalize, especially since they bind to DNA more strongly than **1** and **2** according to their ΔT_m values. The present work, along with a recent report of the nonmutagenicity of **10** (X = H)[18] and several other *N*-methylanthracyclines, suggests that mutagenicity may be lost with any alkylation of the amino nitrogen. Thus, an important biological property can be easily altered by a chemical modification.

Initial attempts to define the structural features responsible for the unique properties of **5g** (X = H) provided the following results. Assuming the steric bulk of the benzyl groups was inhibiting intercalation, the *N*-decyl derivative **3e** (X = H) was prepared in an attempt to approximate the lipophilic character of **5g** (X = H) while retaining the capacity to bind to DNA. As expected, **3e** (X = H) did bind to DNA but had essentially no in vivo antitumor activity. The didecyl derivative **5e** (X = H), which judging by its TLC behavior more closely approximates the lipophilicity of **5g** (X = H), lacked both in vivo activity and DNA-binding capability. Efforts to prepare the dipivalyl derivative **5f** (X = H), which should have approximately the same lipophilicity as **5g** (X = H) but with increased steric hindrance about the 3′-amino nitrogen, were unsuccessful as noted above.

In order to improve on the aqueous solubility of **5g** (X = H; 0.2 mg/mL), an attempt was made to prepare the *N,N*-bis[*p*-(dimethylamino)benzyl] derivative. While this was unsuccessful (vide supra), it should be noted that the solubility of **5g** (X = H) was increased to >36 mg/mL by the use of 10% propylene glycol as the vehicle.

The 13-dihydro derivatives that were obtained as side products are logically the initial in vivo metabolities of the 13-keto compounds, based on the known metabolism of **1** and **2**. Ketone reduction is not a deactivation process and has even been suggested as being an activation step.[19] Thus, it is interesting that the 13-dihydro derivatives all show activity comparable to the activity of the parents.

In summary, the N-alkylated anthracyclines are readily accessible and highly efficacious analogues of the proven antitumor agents adriamycin and daunorubicin. Unexpected alterations in biological properties are observed that suggest interesting alterations in biochemical mechanisms and, more important, possible alterations in therapeutic properties. There are large differences in DNA-interactive properties which are distinct from changes in RNA-interactive properties. There is a consistent loss of microbial mutagenicity with all the compounds tested, but there are both increases and decreases in cardiotoxicity as measured in the Zbinden test. These changes in properties have been attained by a one-step chemical modification. The increased potency of RNA synthesis inhibition indicative of class II properties observed for some of the N-alkylated compounds was previously observed only in natural anthracyclines. Further studies that elucidate the biochemical mechanisms of these compounds may permit the selection of agents in which the antitumor properties are separated from the cardiotoxicity and other side effects. The dibenzyl derivative **5g** (X = H) is of special interest in this regard for its increased antitumor efficacy, decreased cardiotoxicity, and loss of DNA binding and microbial mutagenicity. Further studies of these compounds and their effects are in progress.

Experimental Section

Solvent extracts of aqueous solutions were dried over anhydrous Na_2SO_4. Solutions were concentrated under reduced pressure using a rotary evaporator. Measurements of 100-MHz NMR were performed by Mr. L. Cary using a Varian XL-100 spectrometer in $CDCl_3$, with Me_4Si as an internal standard, unless otherwise stated. Elemental microanalyses were provided by the microanalytical laboratory of Stanford University.

Thin-layer chromatograms (TLC) were obtained on silica gel GF 250-μm plates (Analtech). Preparative layer chromatograms (PLC) were obtained on 20 × 20 × 0.2 cm silica gel 60 F-254 plates (E. Merck). Preparative liquid chromatography was performed on Bio-Sil A, 200–325 mesh, and dry column chromatography on activity III silica gel (ICN), 305 mesh (Bio-Rad), or on a Waters Prep LC/500 system.

***N,N*-Dimethyldaunorubicin Hydrochloride (5a, X = H).** To a stirred solution of 1 (2.80 g, 5.0 mmol) in 2:1 acetonitrile–water (55 mL) was added formaldehyde (3.8 mL of 37% aqueous solution, 50.0 mmol). The solution was stirred at 23 °C for 30 min and added dropwise over 20 min to a stirred solution of $NaCNBH_3$ (0.62 g, 10.0 mmol) in acetonitrile (30 mL). After the addition, stirring was continued at 23 °C for 20 min, and the reaction mixture was diluted with water (100 mL) and extracted with $CHCl_3$ (2 × 100 mL). The extracts were combined, dried, and evaporated. The residue was chromatographed [2.9 × 45.7 cm column of silica gel (150 g), $CHCl_3$ to 20:10:1 $CHCl_3$–MeOH–H_2O] to afford 2.3 g of **5a** (X = H) and 0.18 g of ***N,N*-dimethyl-13-dihydrodaunorubicin (6a, X = H)** as the free bases. The HCl salts were prepared by treating a cold (5 °C) $CHCl_3$ solution of each compound with a molar equivalent amount of methanolic HCl, followed by precipitation with ether, to afford 2.26 g (80%) of **5a** (X = H) and 0.18 g (6%) of **6a** (X = H). **5a** (X = H): IR 3.03 (OH, NH), 5.83 (C=O), 6.14, 6.27 μm (H-bonded quinone); NMR (Me_2SO-d_6) δ 1.21 (d, 3, 6-H_3), 2.00 (m, 2, 2′-H_2), 2.16 (m, 2, 8-H_2), 2.32 (s, 3, 14-H_3), 2.76 (s, 6, NMe_2), 2.89 (m, 2, 10-H_2), 3.42 (m, 1, 4′-H), 3.95 (m, 1, 3′-H), 3.95 (s, 3, OMe), 4.18 (m, 1, 5′-H), 4.91 (br s, 1, 7-H), 5.39 (br s, 1, 1′-H), 7.35 (m, 1, 2-H), 7.76 (m, 2, 1- and 3-H's), 13.12 (s, 1, 11-OH), 13.94 (s, 1, 6-OH); UV–vis λ_{max} (MeOH) 233 nm (ε 38 800), 252 (26 400), 288 (8870), 478 (12 500), 495 (12 500), 530 (6750); $[\alpha]_D$ +241° (*c* 0.06, EtOH); TLC (40:10:1 $CHCl_3$–MeOH–H_2O) R_f 0.38. Anal. ($C_{29}H_{33}NO_{10}$·HCl·1.5H_2O) C, H, N, Cl. **6a** (X = H): IR 2.95 (OH, NH), 6.16, 6.30 μm (H-bonded quinone); NMR (free base) δ 1.28 (d, 3, 14-H_3), 1.38 (d, 3, 6′-H_3), 1.80 (m, 2, 2′-H_2), 2.22 (s and m, 8, 8-H_2 and NMe_2), 2.60 (q, 2, 10-H_2), 3.70 (br s, 1, 4′-H), 3.90 (m, 1, 3′-H), 4.10 (s, 3, OMe), 4.16 (m, 1, 5′-H), 5.30 (br s, 1, 7-H), 5.54 (br s, 1, 1′-H), 7.37 (dd, 1, *J* = 8 and 1 Hz, 3-H), 7.77 (t, 1, *J* = 8 Hz, 2-H), 8.03 (dd, 1, *J* = 8 and 1 Hz, 1-H), 13.20 (s, 1, 11-OH), 13.85 (s, 1, 6-OH); $[\alpha]_D$ +188° (*c* 0.05, EtOH); TLC (40:10:1 $CHCl_3$–MeOH–H_2O) R_f 0.25. Anal. ($C_{29}H_{35}NO_{10}$·HCl·0.75H_2O) C, H, N, Cl.

***N*-Benzyldaunorubicin Hydrochloride (3g, X = H).** Benzaldehyde (8.2 mL, 80.0 mmol) and 1 (2.26 g, 4.0 mmol) were placed in 3:1 acetonitrile–H_2O (116 mL) and stirred at 23 °C for 0.5 h. $NaCNBH_3$ (0.75 g, 12.0 mmol) was added, and stirring was continued for 0.5 h. The reaction was quenched with H_2O (150 mL) and extracted with $CHCl_3$ (3 × 100 mL). The extracts were combined and washed with H_2O (30 mL), and the aqueous washings were extracted with $CHCl_3$ (50 mL). The organic solutions were combined and extracted with cold 0.1 N HOAc (6 × 100 mL). The extracts were combined, immediately basified with $NaHCO_3$, and extracted with $CHCl_3$ (3 × 300 mL). The extracts were combined and evaporated, and the residue was chromatographed (1.3 kg of silica gel; 50 mm × 7 ft dry column, 10:1 $CHCl_3$–MeOH) to afford 1.06 g (51%) of **3g** (X = H) and 0.07 g (7%) of ***N*-benzyl-13-dihydrodaunorubicin (4g, X = H)** as the free bases. The organic solution after the HOAc extraction was dried and evaporated. The residue was chromatographed (560 g of silica gel; 50 mm × 3 ft dry column, 10:1 $CHCl_3$–MeOH) to afford 0.18 g (6%) of ***N,N*-dibenzyldaunorubicin (5g, X = H)** free base. The HCl salts were prepared by treating a $CHCl_3$ solution of each compound with a molar equivalent amount of methanolic HCl followed by precipitation with ether to afford 1.28 g (49%) of **3g** (X = H), 0.16 g (6%) of **4g** (X = H), and 0.16 g (5%) of **5g** (X = H). **3g** (X = H): $[\alpha]_D$ +252° (*c* 0.05, 95% EtOH); TLC (10:1 $CHCl_3$–MeOH) R_f 0.60; NMR (free base) δ 1.38 (d, 3, 6′-H_3), 1.80 (m, 2, 2′-H_2), 2.10 (m, 2, 8-H_2), 2.43 (s, 3, 14-H_3), 3.12 (q, 2, 10-H_2), 3.70 (m, 1, 3′-H), 3.77 (d, 2, PhCH_2), 3.96 (m, 1, 4′-H), 4.08 (s, 3, OMe), 4.17 (m, 1, 5′-H), 5.30 (br s, 1, 7-H), 5.50 (br s, 1, 1′-H), 7.38 (m, 6, 3-H and Ar H), 7.77 (t, 1, *J* = 8 Hz, 2-H), 8.03 (dd, 1, *J* = 1 and 8 Hz, 1-H), 13.30 (s, 1, 11-OH), 13.90 (s, 1,6-OH); IR 2.98 (OH, NH), 5.82 (C=O), 6.14, 6.28 μm (H-bonded quinone). Anal. ($C_{34}H_{35}NO_{10}$·HCl·H_2O) C, H, N, Cl. **4g** (X = H): $[\alpha]_D$ +207° (*c* 0.047, 95% EtOH); TLC (10:1 $CHCl_3$–MeOH) R_f 0.4; NMR (free base) δ 1.35 (d, 3, 14-H_3), 1.38 (d, 3, 6′-H_3), 1.80 (m, 2, 2′-H_2), 2.40 (m, 2, 8-H_2), 3.00 (q, 2, 10-H_2), 3.70 (m, 1, 3′-H), 3.75 (d, 2, PhCH_2), 3.90 (m, 1, 4′-H), 4.08 (s, 3, OMe), 4.17 (m, 1, 5′-H), 5.30 (br s, 1, 7-H), 5.52 (br s, 1, 1′-H), 7.38 (m, 6, 3-H and Ar H's), 7.77 (t, 1, *J* = 8 Hz, 2-H), 8.03 (dd, 1, *J* = 1 and 8 Hz, 1-H), 13.30 (s, 1, 11-OH), 13.90 (s, 1, 6-OH); IR 2.91 (OH, NH), 6.18, 6.31 μm (H-bonded quinone). Anal. ($C_{34}H_{37}NO_{10}$·HCl·1.25H_2O) C, H, N, Cl. **5g** (X = H): $[\alpha]_D$ +282° (*c* 0.048, 95% EtOH); TLC (10:1 $CHCl_3$–MeOH) R_f 0.90; NMR (free base) δ 1.30 (d, 3, 6′-H_3), 1.80 (m, 2, 2′-H_2), 2.15 (m, 2, 8-H_2), 2.43 (s, 3, 14-H_3), 3.15 (q, 2, 10-H_2), 3.75 (s, 4, PhCH_2), 3.80 (m, 1, 3′-H), 3.90 (m, 1, 4′-H), 4.08 (s, 3, OMe), 4.17 (m, 1, 5′-H), 5.30 (br s, 1, 7-H), 5.52 (br s, 1, 1′-H), 7.38 (m, 11, 3-H and Ar H's), 7.77 (t, 1, *J* = 8 Hz), 8.03 (dd, 1, *J* = 1 and 8 Hz, 1-H), 13.0 (s, 11-OH), 13.9 (s, 1, 6-OH); IR 2.90 (OH, NH), 5.82 (C=O), 6.14, 6.28 μm (H-bonded quinone); UV–vis λ_{max}(MeOH) 233 nm (ε 39 800), 252 (27 200), 289 (9000), 478 (12 700), 495 (12 600), 530 (6600). Anal. ($C_{41}H_{41}NO_{10}$·HCl·0.75H_2O) C, H, N, Cl.

***N,N*-Dibenzyldaunorubicin Hydrochloride (5g, X = H).** Benzaldehyde (180 mL, 1.76 mol) was added to a solution of 1 (49.4 g, 87.6 mmol) in 2:1 acetonitrile–water (1.8 L). After stirring at 23 °C for 45 min, the solution was added over a 45-min period to a stirred solution of $NaCNBH_3$ (11.2 g, 0.18 mol) and benzaldehyde (90 mL, 0.88 mol) in acetonitrile (40 mL). The reaction mixture was stirred at 25 °C for 7 days and diluted with water (3 L). This mixture was washed with petroleum ether (16 × 1 L) and extracted with CH_2Cl_2 (4 × 1.5 L). The extracts were combined, washed with H_2O (4 × 750 mL), dried, and evaporated. The residue was divided into four portions, each of which was chromatographed (silica gel, Waters Prep LC 500, 2.5–20% *i*-PrOH in CH_2Cl_2) to afford in order of elution **5g**, **6g**, **3g**, and **4g** (X = H). These were converted to their HCl salts as described above to afford 45.62 g (70%) of **5g** (X = H), followed by 3.6 g (5.6%) of ***N,N*-dibenzyl-13-dihydrodaunorubicin hydrochloride (6g, X = H)**, 0.46 g (0.8%) of **3g** (X = H), and 0.18 g (0.3%) of **4g** (X = H). **6g** (X = H): $[\alpha]_D$ +247° (*c* 0.049, 95% EtOH); TLC (10:1 $CHCl_3$–MeOH) R_f 0.85; NMR (free base) δ 1.35 (d, 6, 6′- and 14-H_3's), 1.5–2.0 (m, 2, 2′-H_2), 2.50 (m, 2, 8-H_2), 3.00 (q, 2, 10-H_2), 3.74 (s, 4, PhCH_2), 3.79 (m, 1, 3′-H), 3.98 (m, 1, 4′-H), 4.11 (s, 3, OMe), 4.17 (m, 1, 5′-H), 5.30 (br s, 1, 7-H), 5.53 (br s, 1, 1′-H), 7.24 (m, 10 H, Ar H's), 7.38 (dd, 1, *J* = 1 and 8 Hz, 3-H), 7.77 (t, 1, *J* = 8 Hz, 2-H), 8.05 (dd, 1, *J* = 1 and 8 Hz, 1-H), 13.36 (s, 1, 11-OH), 13.97 (s, 1, 6-OH); IR 3.0 (OH), 6.17, 6.30 μm (H-bonded quinone). Anal. ($C_{41}H_{43}NO_{10}$·HCl·1.5H_2O) C, H, N, Cl.

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A Phenazine Analogue of Actinomycin D

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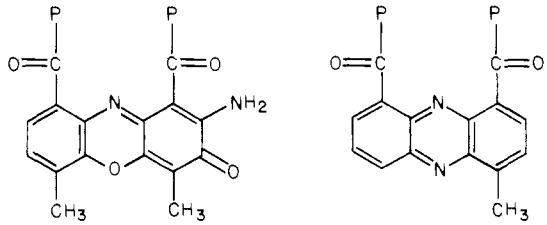
An analogue of actinomycin D (1), in which the phenoxazone chromophore has been replaced by a phenazine, has been synthesized and characterized. Although this compound (2) lacks the 2-amino group and does not possess the quinoid structure of 1, it does bind to DNA, but less tightly than either 1 or the 2-deamino derivative of 1. NMR and CD spectra indicate that the peptide conformations in 2 are approximately as in 1; there was no apparent asymmetry of the two peptide rings. Compound 2 inhibited nucleic acid synthesis in L1210 cell cultures more effectively than does 2-deaminoactinomycin D, but about one-tenth as well as does actinomycin D.

The potent activity of the antibiotic actinomycin D (1)



1

2

P = -Thr-D-Val-Pro-Sar-MeVal—

against several tumors[1-3] is well known; unfortunately the high toxicity of 1 limits its usefulness. Because 1 strongly inhibits DNA-dependent RNA synthesis[4] and also, to a lesser extent, DNA synthesis, its interaction with DNA has been extensively studied, and details of the mechanism of binding to DNA have been elaborated.[5-7] Sobell[6] has argued that hydrogen bonds linking groups on the peptide and sites on the guanine base are responsible for the G-C specificity of 1. Although it has been assumed that the antitumor activity of 1 is due to its inhibition of growth following the intercalative binding to DNA, it is quite possible that the distortions in helical DNA resulting from intercalactive binding are not completely responsible for observed biological effects. For example, Bachur[8] proposes that an intermediate free-radical metabolite may be the active form of 1 that causes DNA damage and cell death.

Although a planar chromophore is a prerequisite for intercalative binding, if the primary role of the chromophore is simply to align the peptide portion of the molecule relative to the DNA helix, chromophores other than that occurring naturally might function as well or better in this role. We have reasoned that an actinomycin analogue in which the pentapeptide lactone rings of 1 have been condensed with an approximately isosteric chromophore should be of value in investigating various possible mechanisms of action. No such analogues with variations of the chromophore nucleus have been reported in the literature, although a number of actinomycins with various substituents in the 2,[9,10] in the 4 and 6, and in the 7 positions of the phenoxazinone nucleus have been prepared.[10] Since phenazines exhibit antibiotic properties, presumably due to intercalative binding to DNA,[11] we selected 2, in which a phenazine residue replaces the phenoxazone system, for synthesis and study to determine the effect of this chromophore substitution on DNA binding and nucleic acid synthesis in cell culture. Some changes in the substitutions on the chromophore of 2 are dictated by synthetic accessibility. However, we have found[9] that loss of the 2-amino group in 1 does not prevent binding to DNA and does not destroy (but does decrease) the in vivo antitumor activity and ability to inhibit nucleic acid synthesis in tissue culture. Additionally, the chromophore 2 lacks the quinoid structure of 1. Unless it is metabolically activated to such a structure, 2 could not participate in the type of oxygen uptake catalysis proposed by Bachur[8] as the biologically important activity of 1. Thus, the more drastic changes in 2 are an important probe of the role of the chromophore in DNA binding and biological activity.

Synthesis. The synthesis of 2 was considered as three efforts: (1) the preparation of the 1,9-dicarboxyphenazine 5, (2) the synthesis of blocked pentapeptide lactone 13, and (3) the condensation of 5 and deblocked 13 to give the final product 2.

At the time this work was initiated, the literature contained no reference to the synthesis of 1,9-dicarboxyphenazine (4) or its derivatives. Breitmaier and Hollstein[12] reported the preparation of 4 and its methyl ester shortly thereafter.

The two 1,9-dicarboxyphenazines 4 and 5 were synthesized by the route shown in Scheme I. Condensation of the anthranilic acid with 2-bromo-3-nitrobenzoic acid[13] was carried out by the method of Flood,[14] using 1-propanol

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