# EFFECTS OF THE STEREOCHEMICAL CONFIGURATION ON THE INTERACTION OF SOME DAUNOMYCIN DERIVATIVES WITH DNA

Franco Zunino, Romolo Gambetta, Aurelio DiMarco,
Giuseppe Luoni and Adriano Zaccara

National Cancer Institute, Via Venezian 1, 20133 Milano, Italy

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Four stereoisomeric daunomycin derivatives, characterized by the absence of the methoxyl group in position 4, have been examined for effects on the thermal denaturation of calf thymus DNA and for their ability to bind to DNA, in order to delineate the antibiotic steric features affecting the binding ability. The major conclusions are: (a) the inverted configuration at positions 7 and 9 markedly decreases the DNA binding; (b) the consequences of the above stereochemical inversion are more critical that the inversion of configuration at position 1 of the amino sugar; (c) in general, the effects on the *in vitro* activity of nucleic acid polymerizing enzymes are consistent with the DNA interaction properties. The structure-activity correlations deduced from these studies are in agreement with earlier findings relating to antitumor activity.

Previous reports from this laboratory have been concerned with the DNA binding properties of anthracycline antibiotics, related to daunomycin and adriamycin (1,2). In an effort to elucidate which factors may affect the DNA binding affinity of these drugs, we have suggested the importance of the structure and the stereochemistry of the amino sugar moiety in the binding process (2). Further studies documented that the inverted configuration at position 1 of the amino sugar resulted in a reduced binding and decreased biological activity (3,4).

In an attempt to further define the relations between the stereochemistry of antitumor anthracycline antibiotics and their ability to bind to DNA, we have undertaken a study of a homologous series of compounds, recently synthesized by Arcamone *et al.* (5), all closely related to daunomycin (Fig. 1). Our interest in 4-demethoxy derivatives of daunomycin stems from the availability of four isomers, including α and β anomers of both 4-demethoxydaunomycin and 4-demethoxy-7,9-bis-*epi*-derivative. The interaction between calf thymus DNA and daunomycin derivatives was studied by melting temperature ($T_m$) of DNA and fluorescence quenching.

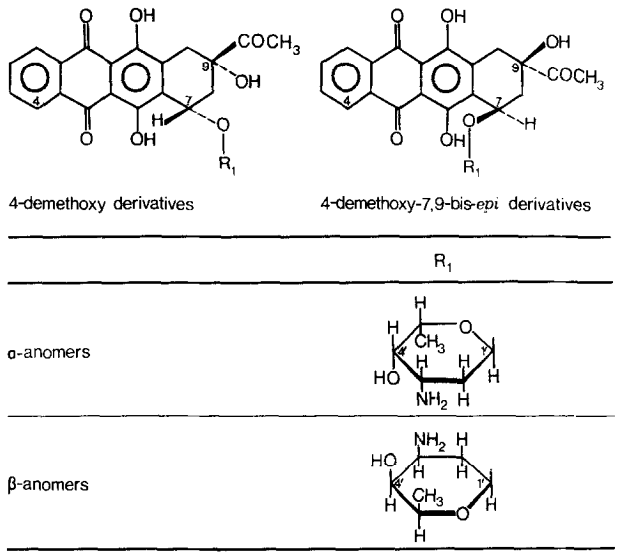


Figure 1. Structure of daunomycin derivatives. As with daunomycin, 4-demethoxydaunomycin is characterized by the 7(S), 9(S) configuration; 4-demethoxy-7,9-bis-*epi*-daunomycin is characterized by the 7(R), 9(R) configuration. The configuration at C-1' of daunomycin, i.e., the stereochemistry of glycosidic linkage, was established as (R) (α glycoside).

## MATERIALS AND METHODS

Materials. Unlabeled and $^{3}H$-labeled nucleotides were supplied by Boehringer Mannheim and by New England Nuclear, respectively. Calf thymus DNA was prepared as described previously (2).

Drugs. Daunomycin and its derivatives were supplied by Farmitalia (Milan). The antibiotics were stored in a desiccator in the dark at 4°. Drug solutions were freshly prepared immediatly before use. The concentrations of the 4-demethoxy derivatives (Mol. wt. 533.5) were determined spectrophotometrically at the $\lambda_{max}$ (485 nm) using a molar extinction coefficient of 9,200 $M^{-1}$ $cm^{-1}$ in the aqueous buffers for all compounds. Their homogeneity has been checked in the laboratories of Farmitalia; the drugs were used without further purification.

Enzyme assays. The bacterial DNA polymerase I assay has been described (6). The enzyme used was a highly purified preparation according to Jovin *et al.* (7). RNA polymerase reaction was carried out as described (8). The RNA polymerase from *E. coli* was isolated by the method of Burgess (9). The incorporation rate was linear during the incubation time for each enzyme. The concentrations of all substrates and templates (or primer-template) were saturating under standard assay conditions.

Binding measurements. The DNA binding of daunomycin derivatives was measured by spectrofluorometry (10). All the antibiotics used were strongly fluorescent, and the method for determining the fraction of bound antibiotic molecules was based on the fact that the fluorescence of the antibiotic is quenched when bound to DNA (11). Binding of anthracycline antibiotics to calf thymus DNA was followed by the decrease in fluorescence at 545 nm with excitation at 470 nm for nucleic acid concentrations of 30 to 1000 μM. Fluorescence measurements were performed with an Aminco Bowman spectrofluorometer.

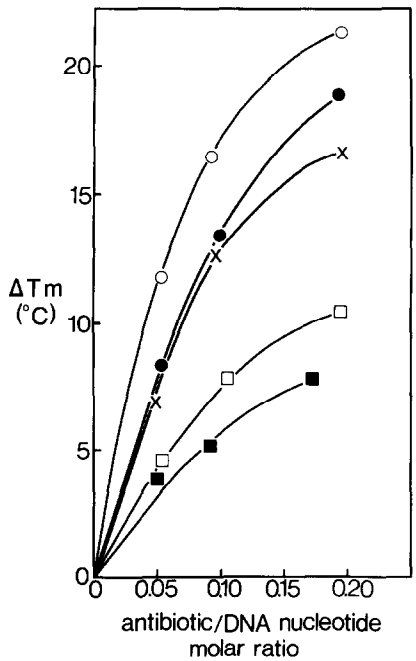


Figure 2. The effect of increasing concentrations of daunomycin derivatives on the $T_m$(midpoint of the thermal denaturation curve) of calf thymus DNA. The study was carried out in 0.01 M Tris-HCl (pH 7.0), 0.01 M NaCl, using 0.1 mM of DNA. The $T_m$ in the absence of antibiotic was found 68.8°. See also reference (2) for details. (X), daunomycin; (O), 4-demethoxydaunomycin; (●), β anomer of 4-demethoxydaunomycin; (□), 4-demethoxy-7,9-bis-*epi*-daunomycin; (■), β anomer of 4-demethoxy-7,9-bis-*epi*-daunomycin.

## RESULTS

The effect of increasing concentrations of the daunomycin derivatives on the $T_m$ of helix-coil transition of calf thymus DNA is shown in Fig. 2. It is evident that all the isomers are capable of binding to DNA, for all of them stabilize the helix to a far greater extent than can be accounted for simply by the increase in ionic strength resulting from their addition. Several interesting observations may be made. (a) Removal of the methoxyl group at position 4 leads to an increased effectiveness in stabilizing the double helix to heat denaturation; (b) the α anomers stabilize the DNA helix to greater extent than do the corresponding β anomers; (c) the derivatives having the 7,9-bis-*epi* -configuration yield a low $\Delta T_m$ increase, as compared to the derivatives with original configuration at positions 7 and 9 of the naturally-occurring

antibiotic.

Further DNA binding studies were carried out using fluorescence quenching technique. The data obtained were analyzed by the Schatchard method (12). Binding parameters were obtained from the slope ($K_{ap}$, the apparent binding constant) and the intercept ($n$, the apparent number of binding sites per nucleotide) of the linear region of the binding curve with the horizontal axis. Although a greater steric hindrance and a lower affinity is expected for the daunomycin-DNA complex, as compared to the 4-demethoxydaunomycin-DNA complex, due to the presence of the methoxyl group in the former, daunomycin binds to DNA ($K_{ap} = 3.3\pm0.8\times10^{6}\ M^{-1}$; $n = 0.18$) as strongly as the 4-demethoxy derivative ($K_{ap} = 2.4\pm0.3\times10^{6}\ M^{-1}$; $n = 0.20$). Binding data for derivatives with unnatural configuration show anomalous binding isotherms, precluding the determination of exact binding parameters. A tendence of the binding isotherm to curve upward at high "$r$" suggests a cooperative binding process (13). However, that the interaction must be substantially weaker is clearly indicated by the different binding properties apparent in Fig. 3. Both the inversion of configuration at C-1' and the inversion at positions 7 and 9 decrease the binding. However, the consequences of the latter stereochemical inversion appear more critical. The following order of decreasing binding is observed: 4-demethoxydaunomycin > β anomer of 4-demethoxydaunomycin > 4-demethoxy-7,9-bis-*epi*-daunomycin > β anomer of 4-demethoxy-7,9-bis-*epi*-daunomycin.

We have compared the influence of these derivatives on nucleic acid polymerase systems as a possible consequence of their interaction with the DNA template (Fig. 4). All derivatives were found to be inhibitors of the enzymes polymerizing the nucleic acids. However, both DNA transcription by *E. coli* RNA polymerase and nucleotide incorporation catalyzed by *E. coli* DNA polymerase I are inhibited to a different extent by the anthracycline drugs. The following order of decreasing effect is observed: daunomycin $\geq$ 4-demethoxydaunomycin > >βanomer of 4-demethoxydaunomycin > 4-demethoxy-7,9-bis-*epi*-daunomycin > > β anomer of 4-demethoxy-7,9-bis-*epi*-daunomycin. As reported for daunomycin

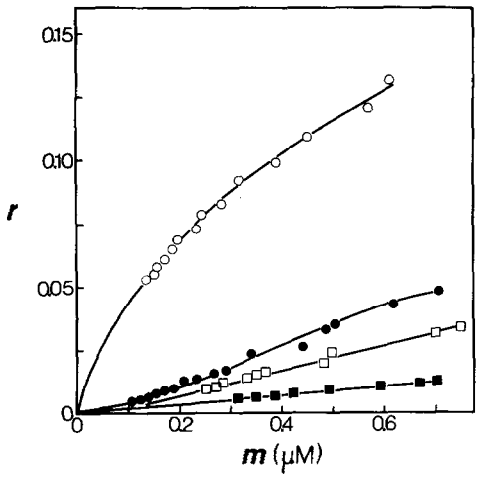


Figure 3. Binding of 4-demethoxy derivatives to native calf thymus DNA. $r$ is the number of drug molecules bound per nucleotide, and $m$ is the free drug concentration. Studies were carried out at 20° in 0.01 M Tris-HCl (pH 7.0), 0.1 M NaCl, and 0.5 mM EDTA. All data were derived from fluorescence quenching studies (see Materials and Methods for details). The same preparation of native DNA was used in all experiments. The designations used are as in Fig. 2.

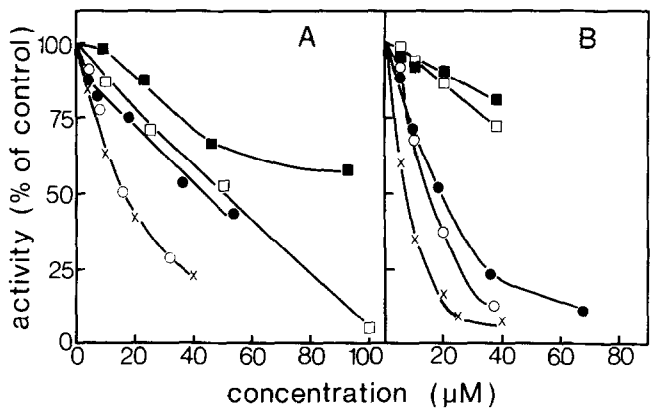


Figure 4. (A) Comparative inhibition of *E. coli* DNA polymerase I by daunomycin derivatives. The assays were done at 37° for 10 min. Control incorporation (no drug added): 250 pmoles [$^3$H]dTMP. (B) Comparative inhibition of *E. coli* RNA polymerase by daunomycin derivatives. Incubation was 5 min at 37°. Control incorporation: 200 pmoles [$^3$H]UMP. The designations used are as in Fig. 2.

and various derivatives (4,14), the 4-demethoxy derivatives having the natural configuration at positions 7 and 9 inhibit RNA polymerase more strongly than

DNA polymerase. This differential effect on the activities of bacterial enzymes is more pronounced in the presence of the β anomer of 4-demethoxy-daunomycin. The behaviour of this derivative may be related to the surprisingly high effectiveness in stabilizing the secondary structure of DNA (Fig. 2). In contrast, it is noted that, comparing the effects on bacterial enzymes under similar assay conditions, the derivatives having the 7,9-bis-*epi*-configuration are somewhat more effective against DNA polymerase at the tested concentrations. Apparently, the data from the inhibition of DNA polymerase are in agreement with those we obtained from the binding experiments; however, with the exception of daunomycin, the effects on DNA transcription are consistent with the effects on $T_m$.

## DISCUSSION

The most caracteristic effects of the interaction with DNA on 4-demethoxydaunomycin, i.e., displacement of the visible absorption spectrum to longer wavelengths, quenching of the fluorescence, are similar to those of daunomycin, which is known to bind to DNA by intercalation (2,15,16). The above observations and the tight binding found for the interaction of 4-demethoxydaunomycin with DNA lead to the conclusion that, at least in this case, an intercalation complex is likely. Therefore, apparently, the bulky methoxyl group at position 4 on the tetracyclic aglycone of daunomycin molecule does not interfere with chromophore/base-pair stacking. Clearly the tightness of binding of the 4-demethoxydaunomycin cannot derive solely from the intercalation of chromophore, but must involve other interactions, presumably on the amino sugar. In this respect, the binding properties of the β glycosides are of great interest because they enable direct assessment of the role of the amino sugar in the complex formation. The amino sugar residue could have an important influence on the orientation of the chromophore within the intercalation site. In support of the suggestion that the amino sugar plays a critical role in dictating the geometry of the antibiotic-DNA complex, the stereochemical inversion at positions 7 and 9 markedly affects the

binding. In this case, it is, nevertheless, possible that a significant contribution to the reduction of binding ability of the 7,9-bis-*epi*-derivatives is afforded by the inversion of configuration at C-9. Some influence of modifications in the C-9 side chain has been reported (2).

In their inhibition of nucleic acid polymerizing enzymes, the 4-demethoxy derivatives show relationships similar to their DNA binding. It is of great interest to note that the above results, obtained with 4-demethoxy derivatives, are consistent with the relative *in vivo* biological activity recently reported (5). Such a correlation is obviously relevant to the factors governing the biological activity and therapeutic effectiveness.

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