The mechanism of inhibition of protein degradation may be via glucocorticoid-mediated stabilization of lysosomal membranes. Pharmacological doses of glucocorticoids given *in vivo* result in hepatic lysosomes which *in vitro* release reduced amounts of their enzymes [17–20].

Department of Biochemistry KENNETH R. CUTRONEO
College of Medicine, DANIEL H. CLARKE
University of Vermont,
Burlington, VT 05401, *U.S.A.*

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The effect of modification at the carbocyclic ring of nogalamycin on the interaction with DNA

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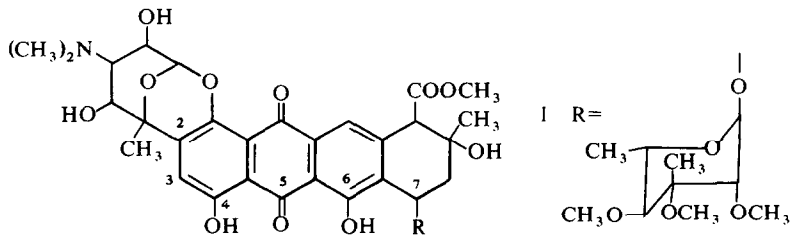
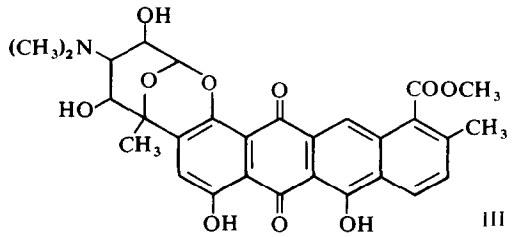
Nogalamycin (I) is an anthracycline antibiotic, but is atypical of this group in that it contains the sugar nogalose rather than an amino-sugar at position 7. Also, the aglycone is in glycosidic linkage at the 1 position with an amino-sugar residue which is additionally bound to the 2 position of the aglycone by a C–C bond [1].



II R=H



III

Nogalamycin has been shown to interact with DNA by intercalation of the chromophore between adjacent base pairs of the helix. That intercalation occurs is shown by the reversal of supercoiling in supercoiled DNA [2], an increase in the Tm of DNA [3] and in the viscosity of DNA solutions [3], and a decrease in the sedimentation coefficient of DNA [3] in the presence of nogalamycin. Also the drug removes kinks from T7 DNA (indicative of stretching of the DNA) [4], it cannot be reduced polarographically when bound to DNA [5], and dichroism studies have shown that the plane of the nogalamycin chromophore is perpendicular to the helix axis when the drug is bound to DNA [6]. The proposal of detailed models for the interaction is however hampered by the lack of a knowledge of the full structure of the drug. Whilst the overall structure has now been elucidated [1], the absolute configuration of the asymmetric centres is not established, only the *relative* configurations of carbons within the carbocyclic ring and within the amino-sugar residue are known. The configurations of the centres in the carbocyclic ring could be 7R, 9R, 10S or 7S, 9S, 10R and the amino-sugar could possess the α-L-or α-D-dideoxyglucose structure [1]. There is no evidence at present to show which of the four possible permutations is the correct stereochemical structure for nogalamycin. Although detailed models for the DNA interaction cannot yet be formulated, an analysis of the effect of structural variation on DNA-binding should lead to a fuller understanding of the binding mechanism.

Nogalamycin has a unique structure in comparison to other anthracycline antibiotics [7]. In most anthracyclines there is an amino-sugar or a disaccharide or trisaccharide unit containing an amino-sugar, at position 7. The amino-group is thought to stabilise the intercalative interaction of the anthraquinone unit of the anthracycline with DNA, by electrostatic interaction with a DNA phosphate group [7]. Nogalamycin, however, bears the amino-sugar at carbons 1 and 2 rather than at carbon 7. Modifications of the carbocyclic ring, for example removal of the 7-substituent, will therefore not lead to a loss of water solubility or to a loss of any stabilising electrostatic interaction of the amino-sugar. Therefore the importance of the carbocyclic ring and its substituents for the nature and affinity of the binding of nogalamycin to DNA can be assessed. Consequently, the interactions of nogalamycin (I), 7-deoxynogalarol (II) and nogalarene (III) with DNA and polyrI.rC have been examined. The latter was chosen as an example of a nucleic acid in the A conformation in contrast to the B conformation of DNA. Because nogalamycin is reported to be base specific [8], binding preferentially to sequences of DNA containing A:T base pairs, some of the tests have been repeated using a natural nucleic acid with the A conformation (*Penicillium chrysogenum* mycophage ds RNA) to examine the validity of using polyrI.rC as a model for a nucleic acid in the A conformation. To purify the sample of ds RNA, it was first treated with deoxyribonuclease I (to remove DNA) and this was followed by chromatography on a Franklin column [9] by the method described previously [10]. This chromatography stage removes the deoxyribonuclease I, and single stranded RNA.

Several spectroscopic tests were used to examine the nature of the interaction of the drugs with DNA and polyrI.rC. For each of the three drugs, six solutions were prepared to contain exactly the same concentration of drug (about 5×10^{-5}M in 0.008 M Tris, 0.05 M NaCl buffer, pH 7.0). One solution contained no DNA and the others contained increasing amounts of DNA such that in the final solution the ratio of DNA phosphate to drug was 20:1. The master DNA solution was prepared by dissolving calf thymus DNA in buffer and was assayed using the figure $\varepsilon(P)_{260} = 6.60 \times 10^3$. Similarly six solutions were prepared for each drug with DNA substituted by polyrI.rC. The master solution of polyrI.rC was prepared by dissolving the polyribonucleotide in distilled water followed by repeated dialysis against the appropriate buffer; the solution was assayed using the figure $\varepsilon(P)_{260} = 4.85 \times 10^3$. The spectra of the drug solutions were then recorded over the range of maximum absorbance of the drug in the visible region of the spectrum. Nogalamycin and 7-deoxynogalarol showed an isosbestic point with DNA but not with polyrI.rC. Nogalarene did not show an isosbestic point with either DNA or polyrI.rC. On binding to DNA, nogalamycin showed a shift in λ_{max} of 13 nm (475–488 nm) and a 28% decrease in extinction; with polyrI.rC only a 1 nm shift (475–476 nm) and an 8 per cent decrease were shown. 7-Deoxynogalarol showed a shift of 8 nm (476–484 nm) and a 25 per cent decrease in extinction with DNA, whereas for polyrI.rC the figures were only 4 nm (476–480 nm) and 4 per cent, respectively. Nogalarene showed no significant shift in λ_{max} or decrease in absorbance with either DNA or polyrI.rC. Whilst spectral shifts cannot be used to determine the nature of the binding without confirmatory evidence from other tests, in all our previous studies with anthracyclines [10–12] large spectral shifts with the presence of an isosbestic point have always, and exclusively, been found for intercalative interactions. This evidence therefore suggests that only the interactions of nogalamycin and 7-deoxynogalarol with DNA are intercalative interactions, none of the compounds appears to intercalate into polyrI.rC and nogalarene also appears not to intercalate into DNA.

Further evidence on the nature of the interaction can be gained by varying the pH of the solution since the phenolic groups of an intercalated anthracycline cannot be ionised when the drug is sequestered within the helix [10]. The free drug solution and the solutions containing the greatest concentrations of DNA and polyrI.rC were adjusted to pH 9 and the spectra compared with the spectra for the corresponding solutions at pH 7. The λ_{max} of nogalamycin shifts by 25 nm (475–500 nm) on increasing the pH from 7.0 to 9.0, in the presence of DNA, however, there is only a 2 nm shift (488–490 nm), whereas with polyrI.rC a shift of 25 nm (476–501 nm) is again seen. This supports intercalation as the mode of interaction with DNA but excludes it as a mechanism of binding to polyrI.rC. The λ_{max} of 7-deoxynogalarol shifts by 25 nm (476–501 nm) on basification, but with DNA the shift is only 8 nm (484–492 nm) indicating intercalation as the mode of interaction. With polyrI.rC a 12 nm shift in λ_{max} was found (480–492 nm) which suggests there is some interaction of the chromophore within the helix, although the mechanism cannot yet be defined. As expected from the previous results, the shifts in λ_{max} on basification of nogalarene in the presence of DNA (30, 494–524 nm) and polyrI.rC (29, 496–525 nm) were equivalent to the shift shown by free drug on basification (28, 497–525 nm). There is therefore no intercalative interaction of nogalarene with either DNA or polyrI.rC.

To further investigate the nature of the interactions, the effect of the nucleic acids on the fluorescence properties of the drug was studied, since often intercalation leads to large changes in the quantum yield of drug fluorescence. Further, if a drug which is rigidly bound to a nucleic acid is irradiated with polarized light, then the fluorescence will be polarized since the drug will not move substantially during the fluorescence lifetime (since the nucleic acid only moves slowly in solution). This is in contrast to unbound drug where Brownian motion will lead to depolarization of fluorescence [10, 11]. Solutions of each drug were therefore prepared to contain about 2.5×10^{-6}M (in 0.008 M Tris, 0.05 M NaCl buffer pH 7.0) and titrated by sequential addition of DNA solution until ratios of 150:1 DNA phosphate to drug were reached (values at 50:1 are quoted in Table 1 alongside the fluorescence as a percentage of that of free drug). Nogalamycin shows an increase in fluorescence polarization and a marked decrease in fluorescence intensity on binding to DNA but these effects are not nearly so marked on interaction with polyrI.rC. Similarly the

Table 1. The effect of DNA and polyrI.rC on the fluorescence properties of nogalamycin, 7-deoxynogalarol and nogalarene

Drug	Free drug		Plus DNA (50:1 DNA/drug)		Plus polyrI.rC (50:1 PolyrI.rC/drug)	
	P*	I†	P*	I†	P*	I†
Nogalamycin	0.13	100	0.21	12	0.17	76
7-Deoxynogalarol	0.12	100	0.17	10	0.16	77
Nogalarene	0.17	100	0.26	33	0.32	98

* P is the fluorescence polarization: $P = F11 - F_{\perp}/F11 + F_{\perp}$ where F_{11} is the fluorescence intensity with both polarisers vertical and $F_{\perp}$ is the fluorescence intensity with the excitation polarizer vertical and the emission polarizer horizontal, the usual correction being made for spurious polarization due to the instrument.

† I is the fluorescence intensity, relative to a value of 100 for free drug.

result for the interaction with DNA of 7-deoxynogalarol are consistent with intercalation as the mechanism of interaction. Although the value of fluorescence polarization for the 7-deoxynogalarol/polyrI.rC interaction is higher than for free drug, the relatively high fluorescence intensity value argues against intercalation as the mechanism of interaction (in support of the previous indications). It should be remembered that an increased fluorescence polarization value will be obtained if the drug binds rigidly to DNA either by intercalation or some other process. The fact that nogalarene shows a marked decrease in the extent of fluorescence depolarization in the presence of DNA and polyrI.rC shows that the rate of molecular movement of this drug in solution is significantly reduced in the presence of nucleic acid. Nogalarene, however, has been shown not to intercalate into either DNA or polyrI.rC and by inference must bind to the exterior of the helix. It should be recalled that all the drugs contain a dimethylamino-group and so would be expected to bind to the surface of DNA electrostatically (the pKa of nogalamycin is 7.45). Unlike other anthracyclines where the amino-sugar link is not rigid, the additional C–C bond to the amino-sugar in nogalamycin immobilises the charged group with respect to the chromophore. An external electrostatic interaction would therefore be revealed by a marked decrease in depolarization of fluorescence as seen here.

An estimation of the stability of the complexes of the drugs with the nucleic acids can be gained from analysis of the effect of the drugs on the thermal denaturation of the nucleic acids. Solutions were prepared containing drug (1.5×10^{-5}M) and nucleic acid (1.5×10^{-4}M) in 0.003 M Tris, 0.018 M NaCl buffer pH 7.0 and the Δ Tm determined by subtraction of the Tm in absence of drug from the Tm in presence of drug, both solutions being determined simultaneously. The results are shown in Table 2, the values obtained for DNA are consistent with previously quoted values [13, 14]. Clearly, there is only a weak interaction of nogalamycin and 7-deoxynogalarol with polyrI.rC compared to DNA. Nogalarene shows only a marginal stabilising effect with both nucleic acids. The results for ds RNA show in these studies that polyrI.rC is a good model for nucleic acids in the A conformation. The significantly increased Tm of DNA in the presence of nogalamycin and 7-deoxynogalarol is additional supporting evidence that these two interactions are intercalative binding, in contrast to the other interactions which have been shown to be nonintercalative. Comparison of the results for nogalamycin and 7-deoxynogalarol show that the latter, which is lacking the nogalose at position 7, has a weaker interaction with DNA than does the parent drug. This suggests that the intercalative interaction of the anthraquinone unit with DNA is stabilised by an additional interaction of the nogalose residue with the helix. This was verified by determination of the affinity constants for the interactions of these two compounds with DNA by spectrophotometric titration. This method was valid since both interactions yielded an isosbestic point. For each of the two drugs, three solutions each of 3 ml and initial concentration 5×10^{-5}M in 0.008 M Tris, 0.05 M NaCl buffer pH 7.0 were titrated with DNA (total addition of 1.0 ml of a 2.75×10^{-3}M solution of DNA in buffer in 22 aliquots of between 20 and 100 μl). The absorbance of the solution was monitored at the wavelength of maximum absorbance for the unbound drug in the visible region of the spectrum against a blank containing an equal amount of DNA. The data were then fitted to an appropriate binding model by the non-linear regression method reported previously [15] with absorbance as the dependent variable and cumulative volume of DNA solution as the independent variable. A one-site model was the simplest model to which the data for nogalamycin could be fitted, and the data analysis yielded values for K and n of 1.432×10^6 M^{-1} and 0.14, respectively. These agree with previously reported values of $K = 1.4 \times 10^6$M^{-1} and $n = 0.15$ (0.2 M BPES buffer) [14]. $K = 0.8 \times 10^6$M^{-1}

Table 2. The difference in the Tm (ΔTm) of nucleic acids in the presence and absence of nogalamycin, 7-deoxynogalarol and nogalarene at a 10:1 ratio of DNA phosphate to drug in 0.003 M Tris, 0.018 M NaCl pH 7.0 buffer

Drug	ΔTm for DNA (°C)	Literature values for DNA (°C)	ΔTm for polyrI.rC (°C)	ΔTm for ds RNA (°C)
Nogalamycin	17.2	16.5* about 20†	3.5	0.5
7-Deoxynogalarol	6.8	6.0*	2.6	
Nogalarene	2.1	4.5*	0.2	1.8

* 10:1 DNA to drug ratio, in 3 mM NaCl, 0.3 mM sodium citrate buffer [13].

† 12:1 DNA to drug ratio in 0.02 M ionic strength buffer [14].

and $n = 0.18$ (0.02 M BPES buffer) [14], $K = 2.5 \times 10^6 M^{-1}$ and $n = 0.14$ (0.01 M Tris buffer) [4] and $K = 6.6 \times 10^6 M^{-1}$ and $n = 0.12$ (0.01 M Tris buffer) [16]. It was not possible to fit the data for the 7-deoxynogalarol/DNA interaction to a two site model, since there were very large standard deviations on the values of K and n obtained and therefore no exact values can be quoted. This is typical of drugs which interact only *weakly* with DNA (K less than 10^5) thus confirming the marked reduction in affinity of binding (seen in the Tm study) which occurs on removal of the 7-substituent of nogalamycin.

In summary, nogalamycin and 7-deoxynogalarol are shown to have an intercalative interaction with DNA, the anthraquinone chromophore must wholly or partially intercalate between adjacent base pairs. This leads to increased stability of the helix to thermal denaturation and the bound drug molecule shows changes in spectroscopic properties consistent with those seen for other anthracyclines which intercalate [10, 11]. In contrast, if the nucleic acid possesses the A conformation, rather than the B conformation of DNA, then an intercalative interaction is precluded. Aromatisation of the carbocyclic ring, as in nogalarene, leads to a loss of the ability to intercalate into the B form and intercalation into the A form is still prohibited. It will be impossible to predict a detailed model for the interaction of nogalamycin with DNA until the full stereochemistry of this drug is resolved. However, it is quite clear that the anthraquinone ring system locates between the base pairs of DNA, that the carbocyclic ring locates within the minor or major groove and that kinking of this ring is essential, a totally planar 4-ring molecule cannot occupy the available binding site. The nogalose sugar unit is actively involved in stabilising the intercalative interaction as deletion results in a lowered binding affinity. Although no information on the role of the amino-sugar has been generated here, it seems probable by analogy with other intercalating drugs, that the amino-group would locate in electrostatic interaction with a phosphate atom. Preliminary skeletal model building shows that despite the bulk of both sugar units, they can locate well within one of the grooves of the helix when the helix is unwound to accommodate the chromophore.

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Department of Pharmacy, University of Manchester, Manchester, M13 9PL, U.K.

TERENCE W. PLUMBRIDGE*
JEFFREY R. BROWN†

* Present address: Sandoz Products Ltd., Feltham, Middlesex, TW13 4EP, U.K.

† Present address: School of Pharmacy, Leicester Polytechnic, P.O. Box 143, Leicester, LE1 9BH, U.K.

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