synthetase and/or stimulators of lipase. The low toxicity and lack of side effects of the lead compound also make this series an attractive one from a therapeutic standpoint. Some analogues of 2-octanone are currently under consideration in our laboratories and will be reported in a subsequent communication.

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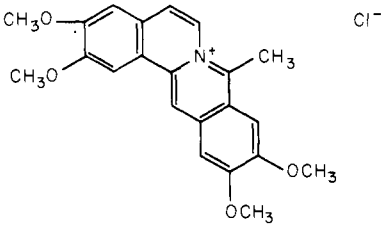
Communications to the Editor

Coralyne. Intercalation with DNA as a Possible Mechanism of Antileukemic Action

Sir:

Coralyne (5,6,7,8,13,13a-hexadehydro-8-methyl-2,3,-10,11-tetramethoxyberbinium chloride, **1**) has been shown to exhibit significant antitumor activity against both P388 and L1210 leukemias in mice.[1] The activity coupled with



1

relatively low toxicity has created an interest in this compound resulting in synthesis of a number of derivatives,[2] as well as a practical large-scale synthesis of coralyne itself.[3] The fused planar cationic aromatic ring system of coralyne is potentially capable of intercalation with DNA in a manner analogous to berberine,[4] daunorubicin,[5] ethidium bromide,[6] and related compounds.[7,8] Zee-Cheng and Cheng[1] have shown that the electronic absorption spectrum of coralyne is perturbed by DNA and that the visible light induced photohydration[9] of coralyne is inhibited in the presence of DNA. They suggested that such an interaction may account for the antileukemic activity of coralyne as has been demonstrated for other antineoplastic agents.[5] Because of the potential importance of coralyne in chemotherapy of neoplasms and the benefits that can arise from an elucidation of its mode of action as an aid in designing more effective derivatives, we have initiated a detailed investigation of the coralyne–DNA complex.

An indication of the strong interaction between coralyne and DNA is shown in Figure 1 by the dramatic shifts induced into the coralyne spectrum upon addition of DNA. The spectra do not display an isosbestic point which suggests that there is more than one bound species of coralyne. Upon addition of DNA to a dilute coralyne solution, the drug spectrum first changes from the characteristic curve of unbound coralyne (Figure 1, curve 1) to a spectrum displaying hypochromism and a red shift at high ratios of coralyne to DNA (Figure 1, curve 2). On continued addition of DNA, the spectrum again shifts slightly to longer wavelengths and an increase in extinction coefficient relative to curve 2 occurs. The spectrum approaches a constant shape on continued addition of DNA and essentially does not change at ratios of coralyne to DNA of less than 0.03 (Figure 1, curve 3). Similar behavior can be obtained by reversing the titration and adding coralyne to DNA solutions provided care is taken to prevent precipitation.

Acridine orange, proflavin, and similar compounds[10,11] also do not display isosbestic points in their DNA spectrophotometric titration curves at low ionic strength and high ratios of drug to DNA. This has been attributed to stacking of the planar aromatic cations on the periphery of the negatively charged deoxyribose phosphate backbone resulting in multiple bound species. As the ratio of these

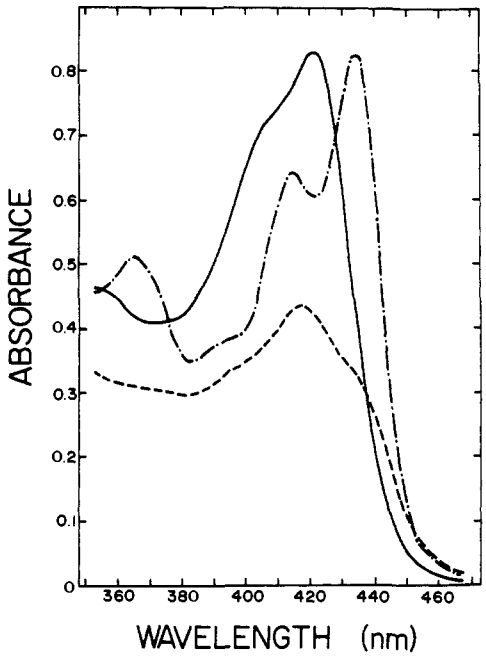


Figure 1. Spectra of unbound coralyne (curve 1, ——), coralyne at a ratio of one drug molecule per nucleotide (curve 2, - - -), and at a ratio of one drug molecule per 33 nucleotides (curve 3, · · · ·). All spectra were recorded in 10-cm lightpath quartz cuvettes using a Beckman Acta V spectrophotometer. Sonicated calf thymus DNA was added from a concentrated stock solution in standard buffer (7.5×10^{-3} M NaH_2PO_4, 10^{-3} M EDTA adjusted to pH 7.0, ionic strength 0.019) to an 8.3×10^{-6} M coralyne solution in standard buffer.

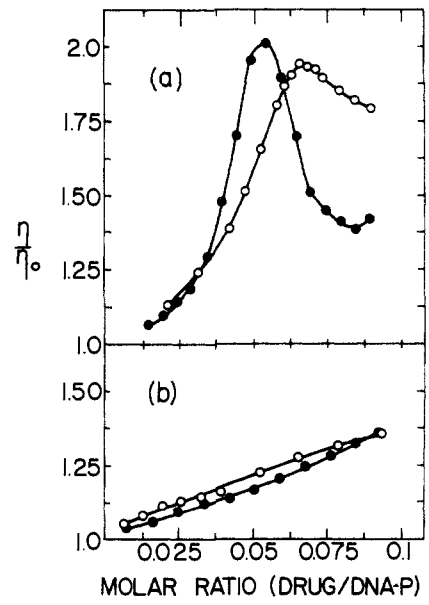


Figure 2. Viscometric analysis illustrating the interaction of coralyne chloride (○) and ethidium bromide (●) with (a) PM-2 bacteriophage closed circular supercoiled DNA and (b) sonicated calf thymus DNA. The reduced specific viscosity ratio (η/η_0) of the DNA-drug complex (η) to DNA alone (η_0) is plotted as a function of the molar ratio of drug added per DNA nucleotide equivalent. Successive aliquots of concentrated drug stock solutions were added with a calibrated microliter syringe to a 1.0-ml DNA solution in the viscometer, and the results were corrected for slight changes in DNA concentration during the titration. The total volume change caused by addition of drug solution was less than 3% of the initial volume. To retard photohydration of coralyne, room light was filtered through yellow plexiglass filters and methyl green was added to the water bath at a concentration of approximately 10^{-5} M. Relative viscosities were determined at 25.0 °C using a Cannon-Ubbelohde semimicro dilution viscometer (Cannon Instruments No. 75-L199) and DNA concentrations ranging between 2.58 and 3.30×10^{-4} nucleotide equivalents per liter. All solutions were in standard buffer.

compounds to DNA is decreased, they also approach a constant spectral shape indicating a single binding mechanism with a predominant binding constant. This bound form at low ratios of compound to DNA has been identified as an intercalated species[11,12] based on the model originally described by Lerman.[7] To determine whether coralyne is intercalated at low ratios of drug to DNA, we have investigated the effects of coralyne on the viscosity of closed circular supercoiled PM-2 DNA and sonicated calf thymus DNA. These two studies, in combination, have provided a conclusive test for intercalation with all compounds studied to this time. The recent finding[13] that a quinoline methanol antimalarial, mefloquine, does not significantly bind to DNA, in spite of its high activity and structural similarity to other quinoline methanols which do intercalate with DNA, illustrates the importance of an experimental investigation of intercalation.

As shown in Figure 2 (a) coralyne removes and reverses the supercoiling of PM-2 DNA in a manner similar to that of the well-characterized intercalating agent ethidium bromide. The maximum in the plot of reduced specific viscosity ratio as a function of coralyne concentration occurs when the supercoils are completely removed from the closed circular DNA. The amount of coralyne required to reach this point is 13% greater than the amount of ethidium bromide required, indicating that the unwinding of the double helix on intercalation of a coralyne molecule is less than that for an ethidium molecule by this amount.[14] Coralyne also increases the viscosity of sonicated calf thymus DNA as predicted for an intercalating molecule[7] and gives results closely paralleling those of ethidium [Figure 2 (b)]. The increase in reduced specific viscosity ratio for the fairly rigid sonicated DNA has been shown to be directly related to the length increase in the double helix caused by an intercalating molecule.[15] The similarity between the coralyne and ethidium titration results with sonicated DNA implies that coralyne elicits a length increase of 3.4 Å per intercalated molecule as has been demonstrated for ethidium.[14] It should be emphasized that the unwinding angle and length increase for an intercalating molecule seem to be independent and can be affected by numerous factors which are still not well understood.[12,14] The increase in viscosity of sonicated DNA and unwinding of closed circular DNA taken together, however, have always provided proof of intercalation. Taken separately neither system is completely satisfactory since nonintercalating molecules are known which will give results similar to an intercalating molecule in one system or the other.

Müller and Crothers[16] demonstrated with actinomine that strong intercalative binding is not a sufficient criterion for medicinal activity, presumably because of the large dissociation rate of its DNA complex. We have shown above that coralyne binds strongly to DNA in an intercalated complex. To determine whether this could be a significant contribution to the antileukemic activity, we have assayed RNA polymerase activity in the presence of coralyne. Coralyne decreased the amount of RNA polymerized by *Escherichia coli* RNA polymerase in the standard assay system described by Wilson et al.[17] but displays significantly less inhibition than the antileukemic

drug daunorubicin. In this system coralyne gives approximately a 20% inhibition of RNA polymerase while daunorubicin gives a 90% inhibition. This finding presents an interesting comparison to in vivo testing results (cf. testing data in references 2 and 18) which indicate that the amount of coralyne required to produce a maximum inhibition of P388 leukemic cells in mice is nearly two orders of magnitude more than the amount of daunorubicin required to give maximum inhibition. At their concentrations of maximum inhibition, however, the two drugs have similar activity.[2,18]

In conclusion, coralyne, at low molar ratios of drug to DNA, can form an intercalated complex with DNA and, as the molar ratio is increased, forms a DNA-induced molecular aggregate stacked along the deoxyribose phosphate backbone. The electronic absorption spectra are quite different for unbound, stacked, and intercalated coralyne molecules. Preliminary results indicate that binding of the stacked form is more sensitive to ionic strength increases than the intercalated species, as would be expected for a complex stabilized primarily by electrostatic interactions.[6] In addition, the RNA polymerase inhibition studies suggest that complexation with DNA could be a factor in the antileukemic activity of coralyne. If so, the low inhibition values relative to daunorubicin could account for the reduced in vivo activity of coralyne relative to daunorubicin. Studies to determine a more exact structure for the stacked and intercalated coralyne complexes, the binding specificity, influence of molecular substitution, and effects of ionic strength are necessary in developing a detailed understanding of how DNA binding might be related to the biological effects of coralyne and are now in progress in our laboratory.

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Book Reviews

Quantitative Analysis by Gas Chromatography. Volume 5. By Joseph Novak. Chromatographic Science Series. Marcel Dekker, New York, N.Y. 1975. ix + 218 pp. 15 × 22.5 cm. $16.75.

This is the fifth volume in a series of monographs in Chromatographic Science and is designed for those who have some experience with gas chromatography. The approach is strongly mathematical. The first five chapters deal with the basic theory. In Chapter 1, the author defines quantitative gas chromatography and expands upon this definition. Chapter 2, which deals with the concentration of the solute in the eluted chromatographic zone, interrelates the column and detector factors by mathematical treatment. Although the function of the detector can easily be imagined as independent of the chromatographic column, the on-line combination of the column and detector yields some new qualities typical of quantitative analysis by gas chromatography. Chapter 3 covers the major considerations in GC detection. The detectors are classified as concentration sensitive/nondestructive (CN), mass sensitive/nondestructive (MN), and mass sensitive/destructive (MD). These detector types are characterized directly in terms of the basic parameters in GC quantitation, i.e., the peak maximum solute concentration, total number of moles of solute, peak height, and peak area. In all cases, the equations relating response to GC parameters are derived and mathematical criteria are also given for the evaluation of the basic characteristics of the various detectors. In Chapter 4, the relationships between peak area and the amount of solute component in the chromatographic band are described. Within this general topic is included the analysis of the signal and response determining parameters of the various detector types, the theoretical and practical aspects involving linearity of response, the derivation of response equations for the various detector types relating the instantaneous amount of substance chromatographed in the sensing element and the detector response, and derivation of equations for the various detector types relating the total amount of the substance chromatographed passing through the sensing element and the time integral of the detector response. In addition, equations are derived relating the effect of an additional auxiliary gas stream on detector response. The molar and relative molar response correction factors are discussed and derived and the analytical significance of uncorrected quantitative parameters of the chromatogram are presented. Chapter 5 is the last chapter dealing with basic theory. It deals with the prediction of relative molar response and shows the possibility of theoretically estimating the relative molar response of the more important