PRE-RESONANCE RAMAN SPECTRA OF ADRIAMYCIN*

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SUMMARY: Pre-resonance Raman spectra have been obtained for aqueous solutions of adriamycin. All bands are attributable to the anthracycline chromophore and are vibrations coupled to the first (20,800 cm^{-1}) electronic transition of the molecule. Both ring modes and vibrations of the carbonyl, hydroxy and methoxy substituents are observed.

INTRODUCTION. Adriamycin is among the most promising of the currently available anti-cancer drugs (1). While very effective as an anti-tumor agent, it is also extremely toxic. The tumor-inhibiting properties have been shown to result from intercalation of adriamycin with DNA, inhibiting normal enzymatic "unwinding" of the DNA molecule (2). Cardiotoxicity and skeletal toxicity of adriamycin are known to be due in part to complexation of calcium, magnesium and other metal cations by the drug (3).

There have been many previous studies of the kinetics and gross mechanisms of binding of adriamycin and related molecules to DNA and, to a lesser extent, to metal ions. There are few investigations of the molecular details of the binding of the drug to either DNA or metal ions. Such studies, however, can provide very useful information for the design of adriamycin derivatives of improved anti-tumor activity or lower toxicity.

The most suitable environment for studying adriamycin interactions with DNA and metal ions is an aqueous solution, where

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Raman spectroscopy provides almost the only useful probe into structural details. As the first part of these studies we report the Raman spectrum of adriamycin in water and deuterium oxide using He-Ne excitation and present tentative assignments of the major bands.

EXPERIMENTAL. Adriamycin (doxorubicin hydrochloride, Adria Laboratories, Wilmington, Del.) was used as received.* Solutions were prepared in distilled water or deuterium oxide. Aqueous solutions were buffered with 0.2 M 2-(N-morpholino)ethanesulfonic acid, adjusted to pH 6.15 with potassium hydroxide. No differences were observed between spectra obtained in buffered or unbuffered solutions.

Raman spectra were recorded with a Spex 1401 double monochromator, equipped with a cooled RCA C31034 photomultiplier. A Spectra-Physics 126 He-Ne laser provided 632.8 nm excitation with 60 mwatt power. Generally, DC detection was employed to obtain adequate offset of the high fluorescence backgrounds encountered.

Spectra were obtained on samples 0.02 - 0.04 M. All spectra were obtained within 24 hours of sample preparation to avoid decomposition.

RESULTS AND DISCUSSION. The Raman spectra of adriamycin in water and deuterium oxide are shown as Figure 1. The noise level encountered precluded obtaining meaningful depolarization ratios, and depolarization data is not presented here.

Examination of the spectra shows that they are preresonance spectra, coupled to the lowest lying π-π^* transition of the anthracycline chromophore. First, the sample concentration

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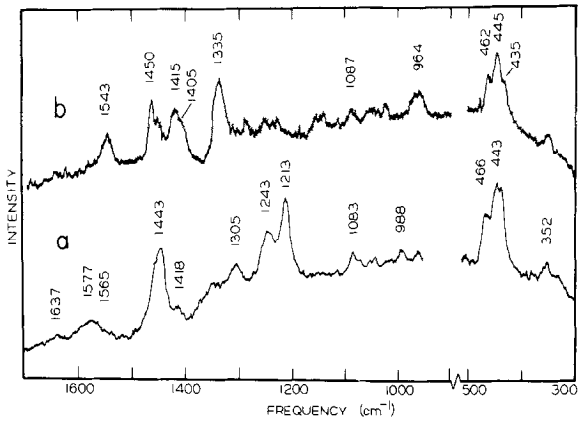

Figure 1. Raman spectra of 0.03 M adriamycin, He-Ne excitation. (a) H_2O solution, pH 6.15 MES buffer. (b) D_2O solution, unbuffered.

range ($\leq$ 0.04 M), is lower than normally required to give fairly intense spectra with a He-Ne laser. Second, no bands attributable to the sugar moiety or the saturated ring of the chromophore are visible. Third, the anthraquinone C=O stretch (IR, 1625 cm^{-1}), which is normally a fairly strong band, is barely visible or absent in these spectra. Finally, the excitation frequency is only some 5000 cm^{-1} below the frequency of the first electronic transition of adriamycin (20,800 cm^{-1}, 480 nm).

Extended Hückel molecular orbital calculations on anthraquinones have confirmed that the first electronic transition involves primarily the hydrocarbon pi-system (4-6). Significantly, the carbonyl pi-system contributes little to this transition. Thus, we would expect to see a preresonance Raman spectrum dominated by aromatic ring modes, with carbonyl stretches weak or absent and vibrations of the saturated ring and the amino-sugar absent.

Although low frequency bands do not change in frequency on

going from H_2O to D_2O solution, it is difficult to assign them unambiguously. Gaziz and co-workers attribute the 490 cm^{-1} band in anthraquinone to carbonyl bending (7), while the 434 cm^{-1} band observed in the fluorescence spectrum of tetrahydroxy-anthraquinone at 4° K, has been assigned to the same vibration (8). It is probable that the 443 and 466 cm^{-1} bands of adriamycin contain large contributions from carbonyl bending, coupled to ring bending modes. With less certainty, we attribute the 352 cm^{-1} band primarily to bending to the carbon-oxygen single bonds of the hydroxy and methoxy side chains. A similar assignment has been made for the 376 cm^{-1} band in the Raman spectrum of 4-nitrocatechol (9).

The weak 1083 cm^{-1} band is attributable to either a methoxy carbon-oxygen stretch or an aromatic carbon hydrogen bend. We can not distinguish between these possibilities on the basis of our data.

Hydroxyanthraquinones typically show two or three bands in the 1200-1300 cm^{-1} region due to coupled phenolic carbon-oxygen stretching and oxygen-hydrogen bending. (8, 10-12). These are observed at 1213-1305 for adriamycin. When D_2O replaces H_2O, these bands disappear and a new strong band at 1335 cm^{-1} appears. Since the coupling of these vibrations depends strongly on the mass of the phenolic hydrogen, substitution by deuterium causes them to disappear.

Although assignment of the new mode is not certain, it is probably predominantly a carbon-oxygen stretch. Internal hydrogen bonding from phenolic to carbonyl groups causes some increase in the nominal carbon-oxygen bond order of the phenolic oxygen due to conjugation around the chelate-type ring formed (4,5,8). Thus, assignment of a band in the 1330 cm^{-1}

region, well removed from the normal phenolic C-O stretch around 1100 cm^{-1}, is reasonable.

The pair of bands at 1418 and 1443 cm^{-1} are assignable to C=C stretches of the aromatic rings. Ring stretching modes are commonly observed in the infra-red spectra of anthraquinones in this region (13,14). The invariance of these bands in D_2O suggests that this assignment is correct.

We are uncertain about the assignment of the band at 1565 cm^{-1}. We do not observe the 1590 cm^{-1} band aromatic C=C stretch found in the infra-red and normal Raman spectra of anthraquinone and most of its derivatives. We believe that the 1565 cm^{-1} band contains a large C=C stretching component, but must also be coupled to modes involving the hydroxy or hydrogen bonded carbonyls because of its shift to 1543 cm^{-1} in D_2O.

As expected, the C=O stretch of the hydrogen-bonded carbonyl (1637 cm^{-1}) is very weak in our aqueous solution pre-resonance spectra. We have been unable to locate this band in D_2O spectra, where it is expected to appear at somewhat lower frequency.

Clearly, it would be useful to study adriamycin interactions with either DNA or metal ions at lower concentrations. Although the adriamycin 480 nm band coincides nicely with the argon ion laser 488 nm line, observation of adriamycin spectra under true resonance Raman conditions is precluded by intense adriamycin fluorescence.

In recent years several fluorescence-rejecting Raman spectroscopic techniques based on time-resolution or stimulated transitions have been proposed. We are presently undertaking measurements of the resonance Raman spectra of low concentrations of adriamycin and its biochemically significant complexes by

coherent anti-Stokes Raman spectroscopy and will report these in a later publication.

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