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Structural elucidation of 4′-epiadriamycin by nuclear magnetic resonance spectroscopy and comparison with adriamycin and daunomycin using quantum mechanical and restrained molecular dynamics approach

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ABSTRACT

The structural and electronic properties of 4′-epiadriamycin, adriamycin, and daunomycin have been studied using density functional theory (DFT) employing B3LYP exchange correlation. The chemical shifts of ^{1}H and ^{13}C resonances in nuclear magnetic resonance spectra have been calculated using Gauge-Invariant Atomic Orbital (GIAO) method as implemented in Gaussian 98 and compared with experimental spectra recorded at 500 MHz. ^{13}C resonances of drugs have been assigned for the first time. A restrained molecular dynamics approach was used to get the optimized solution structure of drugs using inter-proton distance constraints obtained from 2D NOESY spectra. The glycosidic angle C7–O7–C1′–C2′ is found to show considerable flexibility by adopting 156°–161° (I), 142°–143° (II), and 38°–78° (III) conformations, of which the biological relevant structure appears to be the conformer II. The observed different conformations of the three drugs are correlated to the differential anticancer activity and the available biochemical evidence exhibited by these drugs.

The antibiotics, daunomycin and its hydroxyl derivative adriamycin (Fig. 1), are highly active anticancer compounds that have found considerable clinical use [1]. Although both are potent antileukemic agents, adriamycin has an exceptionally broad spectrum of activity, particularly against a variety of solid tumors. The cytotoxic activity of daunomycin and its related compounds has been attributed to intercalation of the anthracycline ring between adjacent base pairs, but other intracellular events such as inhibition of DNA and RNA polymerase and topoisomerase II, enhanced lipid peroxidation and induction of DNA strand breaks have also been suggested to be involved in the mechanism of action of these drugs [1–3]. In view of the pronounced cytotoxicity of these drugs and resistance towards tumor cell lines, there has been continuing search for analogues possessing comparable neoplastic potency [1–3]. One such compound developed is 4′-epiadriamycin (Fig. 1). This molecule differs from adriamycin only by an inversion of the stereochemistry at the 4′-position of the sugar. It is found to be better tolerated due to lesser cardiotoxicity in comparison to daunomycin and adriamycin [4].

Our primary interest is to investigate the precise manner in which 4′-epiadriamycin and its analogues interact with DNA molecule in solution and the optimization of their structure by quantum mechanical calculations. However, in order to understand the drug–DNA interactions, it is essential to study the structural aspects of the drug molecule alone. The X-ray crystal structure of adriamycin and 4′-epiadriamycin has not been reported in literature to the best of our knowledge. The structure of its analogues e.g., daunomycin [5], carminomycin [6], *N*-bromoacetyl daunomycin [7], and daunomycin-butanol [8] have been investigated by X-ray crystallography. These studies have shown that generally C8 atom lies out of plane of the cyclohexane ring A. The conformational properties of daunomycin and its several analogues have also been examined by one-dimensional [9–12] and two-dimensional [13,14] proton NMR spectroscopy in different solvents. It has been reported that several conformers having differences in the relative positions of C8 and C9 atom of ring A may be present in solution [11,12].

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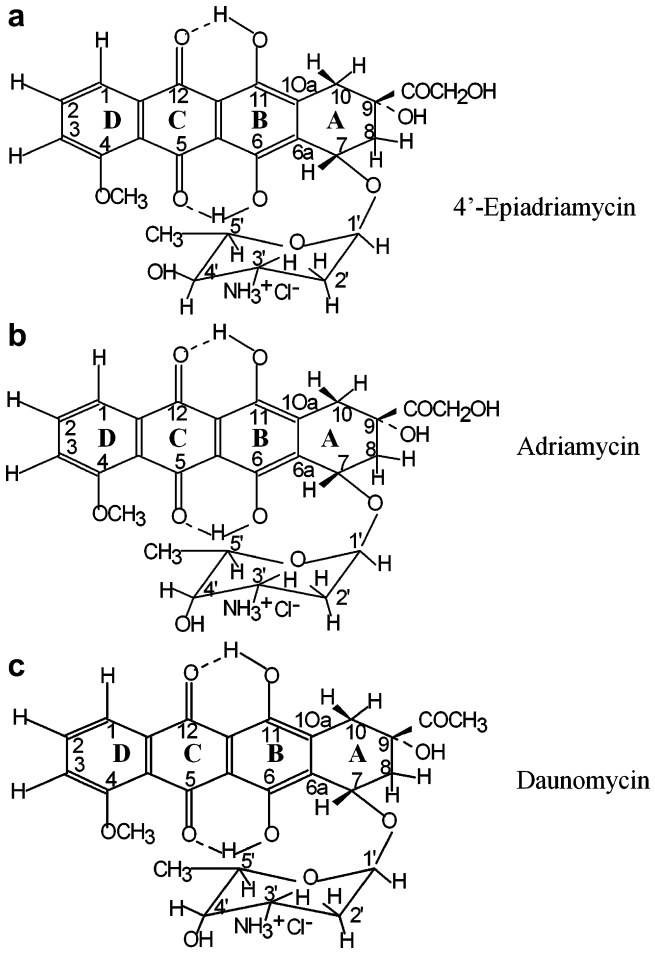

Fig. 1. Chemical structure of (a) 4′-epiadriamycin, (b) adriamycin, (c) daunomycin.

In recent years the NMR chemical shift has become an important tool in the spectral assignment and for rationalizing the experimental chemical shift data. The chemical shift is widely calculated using Gauge-Invariant Atomic Orbital (GIAO[1]) method as implemented in Gaussian 98 package [15]. We have followed the same approach to obtain chemical shifts and other structural parameters for 4′-epiadriamycin, adriamycin, and daunomycin. Several authors [16–21] have used this method successfully for calculating the chemical shifts of heavy atoms, organic compounds, and complexes used in pharmaceutical systems. The popularity of density functional methods and their applications to a broad range of problems of biochemical interest has been growing rapidly each year [22–25]. The most important contributing factor is simply the reliability and cost effectiveness of the calculations.

We have also obtained proton and carbon-13 chemical shifts of 4′-epiadriamycin in D_2O solution using 1D proton NMR combined with 2D homonuclear (^1H–^1H) and heteronuclear (^{13}C–^1H) correlation experiments at 500 MHz. The NMR investigations on closely related drugs, daunomycin and adriamycin, have earlier been reported by our group [13,14]. The conformation of 4′-epiadriamycin, daunomycin, and adriamycin has then been obtained by restrained molecular dynamics (rMD) using torsional angle and inter-proton distance constraints. In addition, we have carried out quantum chemical calculations based on density functional theory (DFT) to compute several molecular parameters and chemical shifts for the three drugs. The results of our restrained molecular dynamics simulations in combination with DFT calculations provide a better insight of the observed features of the measured NMR spectra. To date no such calculations have been reported for the 4′-epiadriamycin, adriamycin, and daunomycin. The structural differences between these drugs are discussed in light of their biological activity.

Materials and methods

NMR Studies

4′-Epiadriamycin was purchased from Calbiochem Pvt. Ltd., San Diego, CA. Deuterium Oxide (D_2O) with isotopic purity 99.9% and other chemicals like Na_2HPO_4 and $NaHPO_4$ etc. used for buffer preparation were obtained from Sigma–Aldrich Chemicals Ltd., St. Louis, MO. Sodium 2,2-dimethyl-2-silapentane-5-sulphonate (DSS), an internal NMR reference was purchased from Merck Sharp and Dohme Ltd., Quebec, Canada. 5.5 mM concentration of 4′-epiadriamycin solution was prepared by dissolving a known quantity of sample in D_2O. This was repeatedly lyophilized and re-dissolved, the process was repeated twice. Final concentration was checked by absorbance measurements at wavelength (λ) = 480 nm using ε = 11,500 M^{-1} cm^{-1}. ^{13}C experiments were recorded with 15 mM concentration of drug sample. The DSS signal was used as internal NMR reference for recording spectra. Experiments were carried out on Bruker Avance 500 MHz spectrophotometer. Typical parameters for one-dimensional NMR experiments were: number of data points 32–64 K, spectral width 5000 Hz, number of scans 64–128, and digital resolution 0.30 Hz/point. Receiver gain was optimized at each instance to obtain the best possible signal to noise ratio. Constant temperature was maintained using the temperature controller unit. 1D-^{13}C experiments were acquired with 32–64 K data points, 2048 number of scans, and 0.7335 Hz/point digital resolution. Double Quantum Filtered Correlation Spectroscopy (dQF COSY), Total Correlation Spectroscopy (TOCSY), Nuclear Overhauser Effect Spectroscopy (NOESY), Hetero-nuclear Single Quantum Correlation (HSQC), and Hetero-nuclear Multiple Bond Correlation (HMBC) experiments were carried out at 298 K in D_2O. Typical parameters for 2D experiments were 1024–2048 data points along t2 dimension, 512 free induction decays in t1 dimension, pulse width ~7.7 μs, spectral width 6000 Hz (^1H)/24,000 Hz (^{13}C), number of scans 64, digital resolution 3.0 Hz/point, and relaxation delay 2.0 s. Data were zero filled in F1 dimension before Fourier transformation. Sine squared bell window function was applied before processing the FIDs.

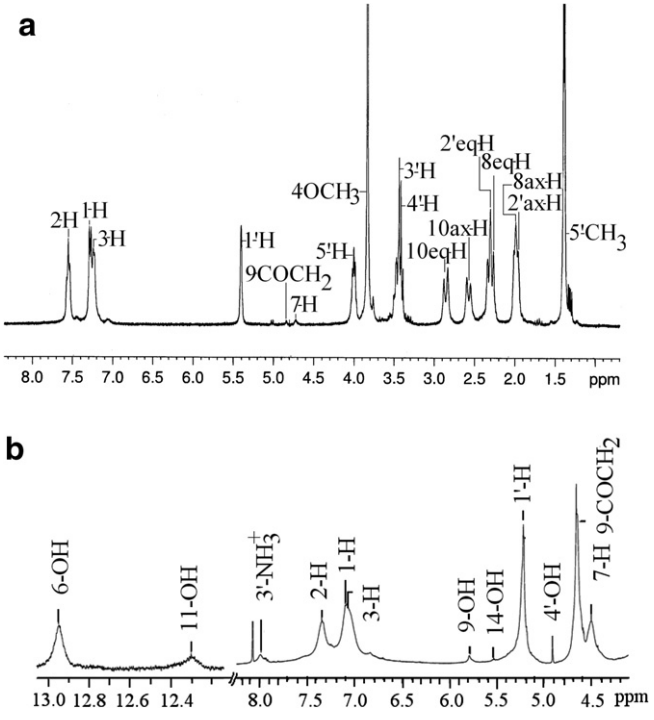

Fig. 2. 500 MHz 1D-proton NMR spectra of 5.5 mM 4′-epiadriamycin in D_2O at (a) 298 K and (b) 275 K (pH 7.0).

[1] *Abbreviation used:* DFT, density functional theory; GIAO, Gauge-Invariant Atomic Orbital; rMD, restrained molecular dynamics; D_2O, Deuterium Oxide; DSS, 2,2-dimethyl-2-silapentane-5-sulphonate; dQF COSY, double quantum filtered correlation spectroscopy; TOCSY, total correlation spectroscopy; NOESY, nuclear overhauser eect spectroscopy; HSQC, hetero-nuclear single quantum correlation, HMBC, hetero-nuclear multiple bond correlation; LYP, lee, yang, and parr's correlation energy; SCF, self consistent field; GTOs, Gaussian type orbitals; TMS, tetramethylsilane.