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ScienceDirect
Journal of Molecular Structure 878 (2008) 139–148
Journal of MOLECULAR STRUCTURE
www.elsevier.com/locate/molstruc

Quantum chemical and nuclear magnetic resonance spectral studies on molecular properties and electronic structure of palmatine molecule

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Received 24 February 2007; received in revised form 30 June 2007; accepted 31 July 2007
Available online 29 August 2007

Abstract

The structural and electronic properties of palmatine have been studied extensively using Density Functional Theory (DFT) employing B3LYP exchange correlation in the gas phase and in the solvent phase. The geometries of the molecule have been fully optimized at B3LYP/6-311G** level of theory. The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) chemical shifts of the molecule have been calculated using the Gauge-Invariant Atomic Orbital (GIAO) method as implemented in Gaussian 98 and 03 versions of program. Tomasi's Polarizable Continuum Model (PCM) was used to evaluate the influence of solvent on the structural parameters and chemical shift values. In addition to DFT we have also examined these properties at different levels of correlation such as MP2/6-31G* only in the gas phase. One (^{1}H, ^{13}C) and two-dimensional (HSQC, HMBC and ROESY) NMR experiments have been recorded at 500 MHz for palmatine molecule in D_2O solvent. All proton and carbon resonances are unambiguously assigned and interproton distances corresponding to the observed NOE contacts have been obtained. Solution structure of palmatine has been determined for the first time by restrained Molecular Dynamics (rMD) using these distance constraints. Comparison of the calculated NMR chemical shifts with the experimental values revealed that DFT method produces good results for both proton and carbon chemical shifts. Further, it is also noticed that the solvent induced structural parameters are very similar to gas phase values but chemical shifts move in the right direction and improve the agreement between theoretical and experimental measurements.

Keywords: Palmatine; NMR; ROESY; Chemical Shift calculations; GIAO; DFT calculations; Restrained Molecular Dynamics

1. Introduction

Palmatine is a quaternary protoberberine alkaloid (Fig. 1) that has been utilized in Ayurvedic and Chinese traditional medicines since long time. It is a tetra-substituted alkaloid with four methoxy groups at C6, C7, C25 and C26 positions. It is found in the roots, rhizomes and stem bark of many species of berberidaceae, fumaraceae, menispermacea and other plant families [1,2]. It exhibits a wide range of pharmacological effects including antimicrobial, antimalarial, anti-inflammatory, antipyretic, hepatoprotective and vasodilatory [3–7] activity. The cytotoxic activity of palmatine to HL-60 leukemic cells has also been well documented [8]. Palmatine has been reported to be effective against experimental tumors by inhibiting the activity of reverse transcriptase [9] and was also found to exert sedative effect by decreasing the levels of catecholamines in rat brains [10]. It acts as photosensitizer by generating singlet oxygen [11]. A few authors have attempted different experimental studies of the X-ray crystal structure [12] and NMR of palmatine and its derivative [13–17]. In recent years Density Functional Theory (DFT) has emerged as a promising alternative to *ab initio* quantum chemical methods. DFT has grown enormously due to its favorable accuracy and computational cost. Huang et al. [18] have performed quantum chemical calculations to study the molecular properties and chemical shift of

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doi:10.1016/j.molstruc.2007.07.046

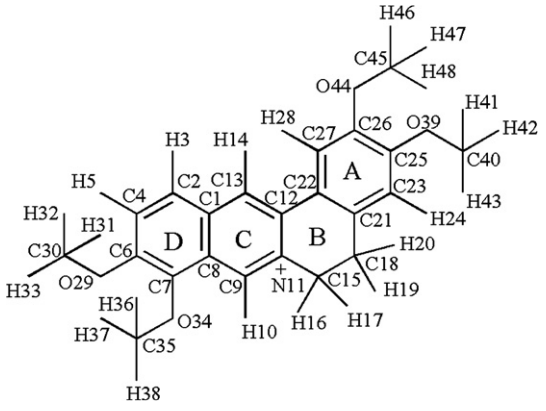

Fig. 1. Structure of palmatine.

another protoberberine, berberine molecule. In the present theoretical investigations, we explore how well DFT predicts structural properties and magnetic properties, in particular chemical shifts which are measured with high accuracy by NMR spectroscopy.

To facilitate the comparison of the DFT results, two *ab initio* methods, Hartree–Fock (HF) self-consistent field and second order Moller–Plesset perturbation theory (MP2) were also used. The chemical shifts are widely calculated using Gauge-Invariant Atomic Orbital (GIAO) method implemented in Gaussian programs [19]. Several authors [20–23] have used this method successfully for calculating the chemical shifts of heavy atoms, organic compounds and complexes used in pharmaceutical systems. We have also followed the same approach to obtain the chemical shifts along with other structural parameters. The polarization induced by the dielectric media around the molecule distorts the electronic wavefunction, and all molecular properties in solution must differ to some extent from their gas phase properties. We, therefore choose the Polarizable Continuum Model (PCM) developed by Tomasi and coworkers [24,25] to describe the bulk solvent. In addition to theoretical investigations, we have taken one-dimensional proton and carbon (^{1}H, ^{13}C) NMR spectra and combined it with two-dimensional homonuclear (^{1}H–^{1}H) and heteronuclear (^{13}C–^{1}H) correlation measurements to obtain the chemical shifts and interproton distances in D_2O solvent. We have for the first time obtained the solution structure of palmatine by restrained Molecular Dynamics (rMD) simulations using interproton distance constraints. The results of our rMD simulations in combination with DFT and *ab initio* molecular calculations provide a better insight of the observed features of the NMR spectra and chemical environment of the solution structure. Here we have examined two aspects of this molecule: (i) structural comparison of the X-ray structure of palmatine with quantum chemical calculations and restrained Molecular Dynamics (rMD) optimized structure. (ii) Comparison of experimental ^{1}H and ^{13}C NMR chemical shifts of palmatine with calculated results both in the gas and the solvent phase using Gauge-Invariant Atomic Orbital (GIAO) method.

2. Experimental

2.1. NMR spectra of palmatine

Palmatine was obtained commercially from Sigma Chemicals Co. Ltd, USA. The sample was prepared by dissolving 6 mg of palmatine in 600 μl of D_2O (concentration 25.78 mM). All the NMR spectra were recorded on a Bruker Avance 500 MHz FT NMR spectrometer at Central NMR Facility of Indian Institute of Technology, Roorkee. Typical parameters for one-dimensional NMR experiments were: pulse width ~7.7 μs, no. of data points = 32 to 64 K, spectral width 5000 Hz, number of scans = 32–64, relaxation delay 1.0 s and digital resolution 0.08 Hz/point. The typical parameters for the 2D Rotating frame nuclear Overhauser Enhancement Spectroscopy (ROESY), Hetero Nuclear Single Quantum Coherence (HSQC), and Hetero Nuclear Multiple Bond Correlation (HMBC) 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 (^{1}H)/24,000 Hz (^{13}C), number of scans 64, digital resolution 3.0 Hz/point and relaxation delay 1.0 s. A mixing time of 300 ms was used for ROESY experiments. A constant temperature of 298 K was maintained all through by using temperature controller.

2.2. Restrained Molecular Dynamics simulations

For restrained Molecular Dynamics (rMD) simulations, initial structures of palmatine have been built using builder module in MOE (Molecular Operating Environment, Chemical Computing Group, Canada) software. This molecule is subjected to 100 steps each of Steepest Descent and Conjugate Gradient and 200 steps of Truncated Newton Raphson methods of minimization to remove any internal strain due to short contacts in starting structures using MMFF94 force field [26,27]. A dielectric constant of 1 was fixed for restrained Molecular Dynamics (rMD) simulations in vacuum. Cross-peaks in the ROESY spectra were integrated using TOPSPIN software and intensities were translated into interproton distances by using formula $I = k/r^6$ (I = volume integral of the peaks, r = interproton distance, k = constant). This method is based on spin pair approximation in which spin diffusion is not taken into account [28]. The interproton distance H3–H5 = 2.45 Å is taken as reference distance. A range of ±0.2 Å was provided to account for any error in integrals. A total number of 10 distance restraints have been used in the structure calculation for the molecule. Pseudo atom correction was used for methyl and other equivalent protons. Conformational search was performed by following simulated annealing restrained Molecular Dynamics protocol. The molecule was heated to a temperature of 800 K in steps of 100 K and equilibrated at this temperature. Molecular Dynamics simulations were carried out for 250 ps at 800 K during which 100 structures were saved at regular intervals. Each of them was then slowly cooled at 300 K in steps of 100 K.