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Molecular characterization of yohimbine hydrochloride using vibrational spectroscopy and quantum chemical calculations

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Abstract

In this work, we have performed the extraction of yohimbine hydrochloride ($C_{21}H_{27}ClN_2O_3$) (YHCl). The optimized geometry, total energy, potential energy surface and vibrational wavenumbers of YHCl have been determined by using *ab initio* Hartree–Fock (HF) and density functional theory (DFT/B3LYP) method with 6-311++G(d,p) basis set. The calculated wavenumbers are scaled by a proper scaling factor. A selected number of vibrational assignment is provided for the observed Raman and IR spectra.

Keywords: YHCl; vibrational spectroscopy; ESP; *ab initio* and DFT calculations

1. Introduction

Yohimbine, an indole alkaloid is the active ingredient obtained from the bark of various tropical trees such as *Pausinystalia yohimbe (*formarly *Corynanthe yohimbe)*, a tall evergreen tree indigenous to southern African countries, and from the Indian snake root *Rauwolfia serpentina* [1,2]. Yohimbine hydrochloride (17α – hydroxy –yohimbane 16α – carboxylic acid methyl ester hydrochloride), a standard form belongs to an orthorhombic system, probable space group $P2_12_12_1$ having lattice parameters, a = 11.54Å, b = 24.88Å, c = 7.00Å and z = 4 [2]. It has been known as an aphrodisiac compound [2,3] and used traditionally since before the last century, even in the crude form by the African natives, and promoted for erectile dysfunction, weight loss (by releasing norepinephrine and blocking alpha-2 receptors) and depression (by blocking an enzyme called monoamine oxidase) [4-6]. It is used in tablets, capsule and tincture form for increased serumtesterone levels, muscle growth and strength, weight-loss, reduce fatigue in AIDS patients and libido enhancer [7]. Also it has been reported as traditional medicine to treat high blood pressure, chest pain, age- related cognitive disorders and obesity [8].

As the literature survey reveals neither Raman and IR spectra nor quantum chemical calculations for YHCl molecule have been reported so far, hence the present work was undertaken to study the vibrational spectra with quantum chemical calculation with greater accuracy. Although X-ray diffraction method is one of the most frequently applied techniques for structural characterization of pharmaceutical compounds but the use of vibrational spectroscopy is also gaining increasing attention. X-ray diffraction techniques are sensible to the long-range order while vibrational spectroscopy (IR [9] and Raman [10]) is applicable to the short-range structure of molecular solids.

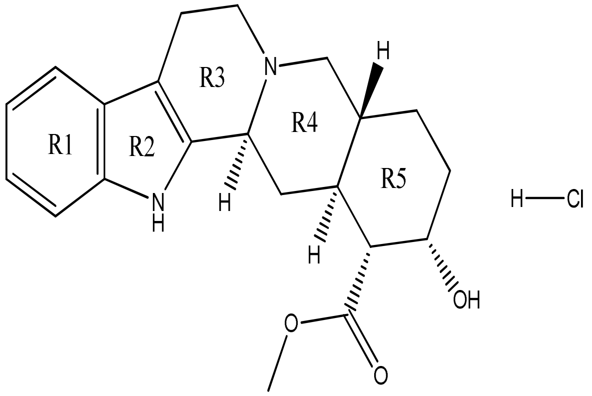

Fig. 1: Crystal structure of YHCl.

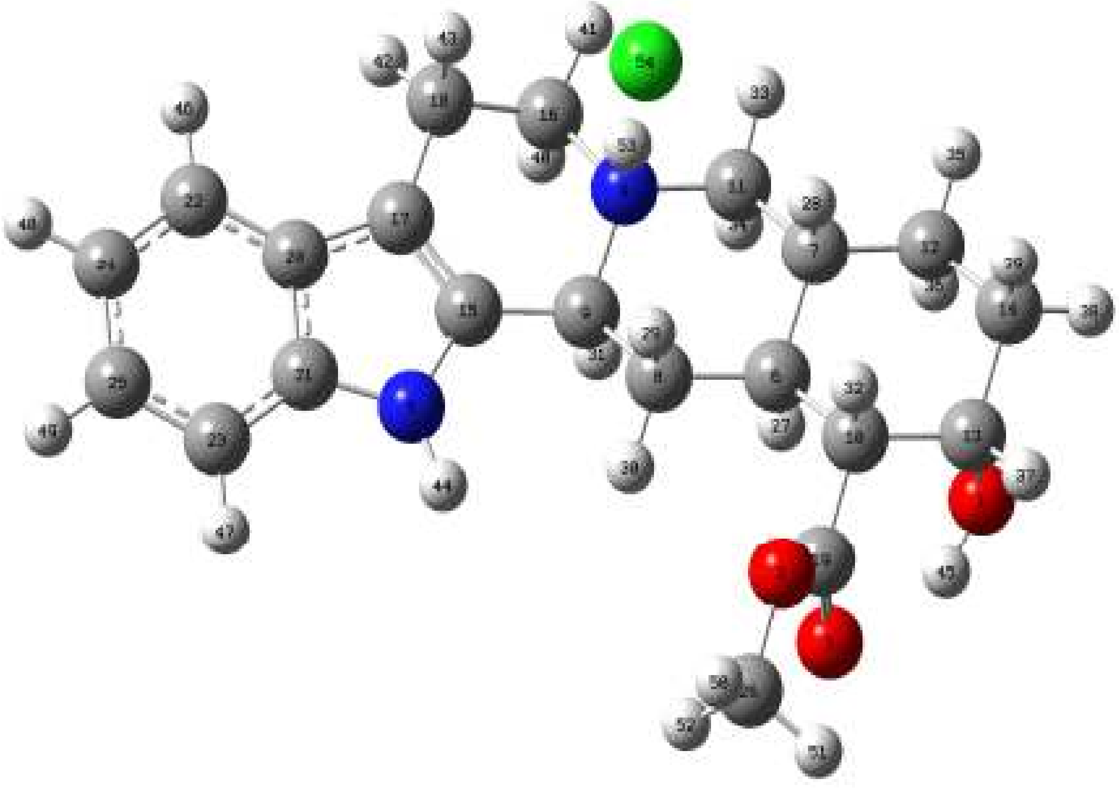

Fig. 2: Optimized structure of YHCl.

Furthermore, we interpreted the calculated spectra in terms of potential energy distributions (PED) and made the assignment of the experimental bands due to PED analysis results. Information about the geometry and structure of the molecule with their electrostatic potential surfaces should help in understanding the structure-activity relationship. Figure 1 shows the crystal structure of the YHCl molecule.

2. Materials and Methods

2.1 Experimental Methods

The whole plants of *Rauwolfia serpentine* Benth. (Apocynaceae) were collected from Lucknow, Uttar Pradesh, India and identified by the Botany Division of Central Drug Research Institute, Lucknow, India. The plant material (2.4 Kg) was air-dried, powdered and percolated with ethyl alcohol (6 x 4 lt) at room temperature. The combined percolate was concentrated under reduced pressure below 40^o to give the viscous mass. The material was partitioned between 2% tartaric acid and hexane. The aqueous solution was re-extracted with hexane (4 x 250 ml), basified with solid Na_2CO_3 (pH 7.5) and extracted with benzene (6 x 250 ml). The combined benzene layer was dried over anhydrous Na_2SO_4 and solvent removed under reduced pressure to give a residue - I. The aqueous solution left after extraction with benzene was further extracted with methylene chloride (5 x 250 ml). The combined methylene chloride layer was dried over anhydrous Na_2SO_4 and solvent removed under reduced pressure to give a residue -II. Both the residues I and II were mixed to afford crude alkaloidal mixture (4.0 g) which was subjected to column chromatography over neutral alumina. The column was successively eluted with hexane, hexane: benzene (20:80), benzene, benzene - ethyl acetate, v/v (98:2), (95:5), (90:10), (85:15), (50:50), (25:75), ethyl acetate, ethyl acetate-methanol (95:5) and methanol. Elution was monitored by thin layer chromatography (tlc). A total of 190 fractions, 100 ml each, were collected and mixed on the basis of tlc. The fractions 42 – 75 eluted from benzene-ethyl acetate (98:2), (95:5) and (90:10) were mixed and solvent removed. The crude product was subjected to preparative tlc (plates: SiO_2 GF_{254}; solvent: chloroform – methanol, 95:5; double run). The major band on the plates was scraped, extracted with chloroform – methanol (3:1), the solvent removed under reduced pressure to give a pure compound (8.4 mg), converted to its hydrochloride, crystallized from methanol - ether, m.p. 299.5^0 [301^0][11,12]. The compound was identified as yohimbine by a direct comparison with an authentic sample procured from Sigma Chemical Company, USA.

An excitation laser of wavelength 514 nm was emitted from an Argon ion laser source and a power of 12 mW was used to record the vibrational spectra using an efficient visible micro Raman setup at room temperature. The scattered Raman light was collected in a back scattering geometry using a microscope objective (ULW x50). The scattered light was dispersed using a monochromator with 1200 grooves/mm diffraction grating, and an entrance slit width of 200 micrometer. The Raman signals were detected using liquid nitrogen cooled charged coupled device (CCD) with an optimal sensitivity in the visible range. The total exposure time for each sample was 5 sec and averaged over five accumulations.

Infrared spectra of YHCl were recorded on a Bruker TENSOR 27 FT-IR spectrometer with a spectral resolution of 4 cm^{-1} in the region 300-4000 cm^{-1}. The KBr pellets of solid samples were prepared from mixtures of KBr and the sample in 200:1 ratio using a hydraulic press. Multi-tasking OPUS software was used for base line corrections.

2.2 Computational Methods

Geometry optimization was performed as the first task of computational work for the YHCl molecule. The experimental geometric parameters from X-ray diffraction data [2] of YHCl molecule were used as the initial parameters for the theoretical calculations. The electronic structure and optimized geometry of the molecule were computed by *ab initio* Hartree-Fock (HF) and DFT (density functional theory) using Gaussian 09 [13] program package employing 6-311++G(d,p) basis set based on Becke's three-parameter (local, non-local, Hartree-Fock) hybrid exchange functional with Lee-Yang-Parr correlation functional (B3LYP) [14].The basis set 6-311++G(d,p) augmented by d polarization functions on heavy atoms and p polarization functions on hydrogen atoms as well as diffuse functions for both hydrogen and heavy atoms were used. The optimized structural parameters were used to calculate the absolute Raman intensities and infrared absorption intensities in the harmonic approximation at the HF and DFT levels. The positive values of all the obtained 156 wavenumbers confirm the stability of optimized geometry. For analyzing the normal a complete set of 156 internal co-ordinates was defined using Pulay's recommendations [15]. The vibrational assignments of the normal modes were proposed on the basis of the PED calculated using the program GAR2PED [16]**.** Raman and infrared spectra were simulated using a pure Lorentzian band profile (fwhm = 8 cm^{-1}) using indigenously developed software. Visualization and confirmation of the calculated forms of the vibrations were done using the CHEMCRAFT program [17].

3. Geometry Optimization and Energies

Initial geometry taken from the X-ray diffraction data [2] of YHCl was minimized without any constraint to the potential energy surface and the optimized structural parameters were used in the vibrational frequency calculation to characterize all stationary points as minima. The equilibrium geometry has been determined by the energy minimization. The optimized structure of YHCl molecule is shown in Figure 2. The relative energies of the molecule are calculated employing *ab initio* HF and DFT functional (B3LYP). The DFT includes some electron correlation effects and hence the calculations with this method are better than the HF approach. The energy calculated by DFT (-1612.2632515 Hartree) is lower than the one calculated by HF (-1612.2607923 Hartree).

The optimized structural parameters (bond lengths, bond angles, dihedral angles) of YHCl have been compared with the experimental one. The difference between experimental and calculated values of bond-lengths is not more than 0.04Å, both in DFT and HF methods. The bond angles differ by not more than 2.5^o except the angles, C19-O2-C26 and O1-C13-C10 which differ by $4.82^o/5.82^o$ and $5.64^o/5.64^o$ in the DFT/HF respectively. Similarly the dihedral angles differ by not more than 4.9^o except the angles C6-C10-C19-O2 and C6-C10-C19-O3 differ by $6.83^o/6.83^o$ and $6.34^o/6.34^o$ in the DFT/HF respectively.

4. Results and Discussion

4.1 Molecular Electrostatic Potential

The molecular electrostatic potential (ESP) of a molecule at a point in space around it gives an indication of the net electrostatic effect produced by the total charge distribution (electrons + nuclei) of the molecule. The molecular ESP of YHCl molecule is shown in Figure 3. It correlates the total charge distribution with dipole moments, partial charges, electro-negativity and site of chemical reactivity of a molecule. ESP provides a visual method to understand the relative polarity of a molecule and serves as a useful quantity to explain hydrogen bonding, reactivity and structure-activity relationship of

molecules including biomolecules and drugs [18]. Different colours represent the different values of the electrostatic potential at the surface of a molecule. In general, red colour represents the regions of most negative electrostatic potential (possess electrophilic property), blue represents the regions of most positive electrostatic potential (possess nucleophilic property) and green represents the regions of zero potential. Potential increases in the order red < orange < yellow <green < blue.

The electrostatic potential has been used primarily for predicting sites and relative reactivities towards electrophilic attack and in studies of biological recognition and hydrogen bonding interactions [19]. In order to predict the reactive sites for electrophilic and nucleophilic attacks of the title molecule, such electrostatic potential surfaces have been plotted for molecules in 6-311++G(d,p) basis set using the computer software GAUSSVIEW ver.09 [14] as shown in Figure 3. In YHCl molecule, the regions near

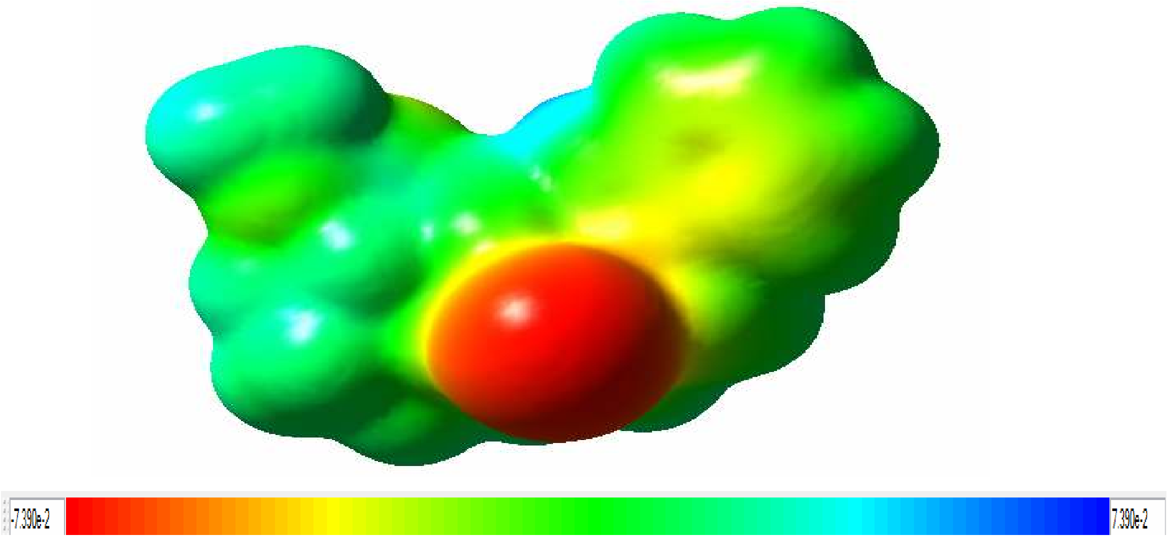

Fig. 3. Molecular electrostatic potential mapped on the isodensity surface in the range from $-7.390*10^{-2}$ (red) to $+7.390*10^{-2}$ (blue) for YHCl.

the chlorine atom of hydrochloride groups as well as those of hydroxyl groups are most negative, while the regions near hydrogen of hydroxyl groups are the most positive regions.

4.2 Vibrational Assignments

The total number of atoms in a molecule is 54, hence gives 156 (3N-6) normal modes of vibrations. All the 156 fundamental vibrations of the YHCL molecule are both Raman and IR active. The vibrational spectrum is mainly determined by the modes of the free molecule observed at higher wavenumbers, together with the lattice (translational and vlibrational) modes in the low wavenumber region. All the vibrational bands have been assigned satisfactorily. The assigned wavenumbers of the selected vibrational modes calculated at the HF and B3LYP level with the basis set 6-311++G(d,p) along with their PED are given in Table.

Since the vibrational wavenumbers obtained from the DFT calculations are higher than the experimental wavenumbers, they were scaled down by the wavenumber linear scaling procedure (WLS) $[v_{obs}/v_{cal} = (1.0087 - 0.0000163 \text{ x } v_{cal})\ cm^{-1}]$ of Yoshida *et al.* [20] and a comparison was made with the experimental values. However there are different scaling factors, but the vibrational wavenumbers calculated uniformly scaled with only one scaling factor [21] are often in good agreement the observed ones. All the calculated vibrational wavenumbers reported in this study are the scaled values.

In the ring R1, the CH stretching of the ring is calculated in the region 3052-3027 cm^{-1} and match well with the values 3057, 3031 cm^{-1} in the Raman and 3087 and 3066 cm^{-1} in the IR spectra. The in-plane deformations of CH group are calculated to be 1453, 1384 and 1238 cm^{-1} and reported at 1452, 1383 and 1245 cm^{-1} in the Raman and at 1452 and 1234 cm^{-1} in the IR spectrum, respectively.

In the ring R2, the N5H stretching vibration is calculated to be 3481 cm^{-1} and observed as high intense peak at 3514 cm^{-1} in the IR spectrum as shown in the Figure 4.

In the ring R3, weak asymmetric stretching mode of $C11H_2$ is calculated at 2972 cm^{-1} and assigned to the peak at 2965 cm^{-1} in the Raman and 2960 cm^{-1} in the IR spectrum. Symmetric stretching vibration

Table: Some selected calculated and experimental wavenumbers (cm^{-1}) of YHCl.

Unscaled	Scaled		Raman	IR	Potential energy distribution
DFT	HF	DFT			
3838	3631	3631	-	3670	O1H - stretching
3669	3481	3481	-	3514	N5H - stretching
3190	3052	3052	3057	3087	CH - stretching
3179	3042	3042	-	3066	CH - stretching
3169	3033	3033	3031	-	CH - stretching
3163	3027	3027	-	-	CH - stretching
3158	3023	3023	-	3024	CH_3 - asym stretching
3125	2993	2993	2989	-	CH_3 - asym stretching
3102	2972	2972	2965	2960	$C11H_2$ –asym stretching
3052	2927	2927	-	2935	CH_3 - sym stretching
3034	2910	2910	2911	-	$C11H_2$ – sym stretching
3017	2895	2895	2894	2887	(C6H +C7H) - stretching
3006	2885	2885	2876	2883	(C7H + C6H) - stretching
1785	1748	1748	1754	1718	C=O - stretching
1478	1455	1456	1453	1454	C18H2 - deformation
1476	1453	1453	1452	1452	CH - in-plane deformation
1424	1403	1403	1408	1408	HC13O1 - deformation
1403	1384	1384	1383	-	CH - in-plane deformation
1253	1238	1238	1245	1234	CH – in-plane deformation
1176	1163	1163	1165	-	C13O1H45 - deformation

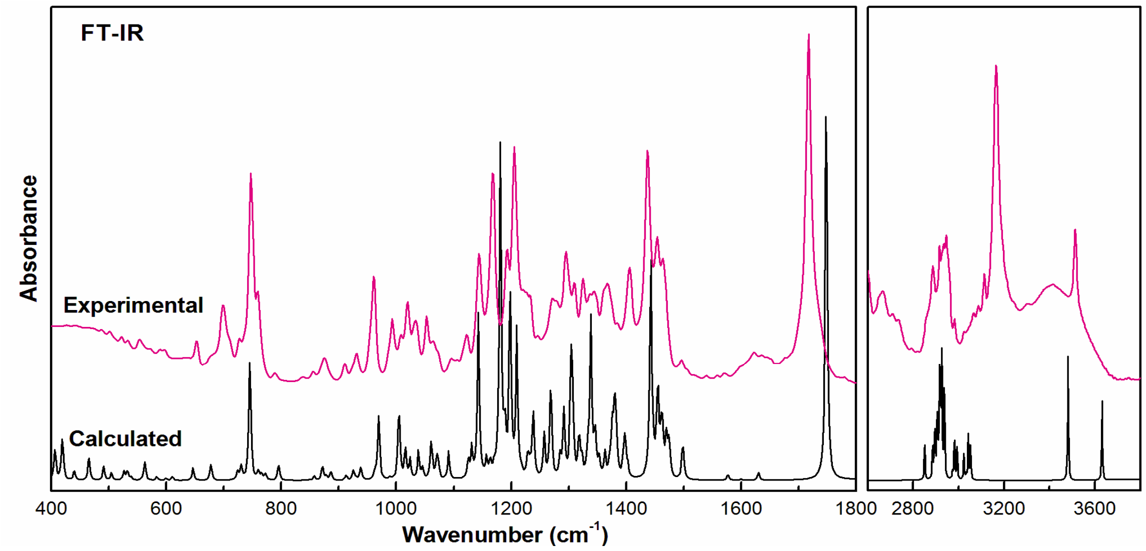

Fig. 4: Experimental and calculated (scaled) IR absorption spectra of YHCl in the Region 400-1800 and 2600-3800 cm^{-1}

of this mode is calculated to be 2910 cm^{-1} and reported at 2911 cm^{-1} in the Raman spectrum. The mixed vibrations of C7H and C6H stretching modes are calculated to be 2885 / 2895 cm^{-1} and observed at 2876/2894 cm^{-1} in the Raman and at 2883/2887 cm^{-1} in the IR spectrum, which match well with the experimental results.

In the ring R4, the stretching vibration of alcoholic O1H group is calculated at 3631 cm^{-1} and observed to the weak band in the IR spectrum at 3670 cm^{-1}. The O1H deformations are calculated to be 1403/1163 cm^{-1} and reported at 1408/1165 cm^{-1} with very weak band in the Raman and at 1408 cm^{-1} with a strong band in the IR spectrum.

The asymmetric stretches of CH_3 group are calculated to be 3023 and 2993 cm^{-1} and reported at 2989 cm^{-1} with strong intensity in the Raman and with weak intensity at 3024 cm^{-1} in the IR spectrum. The CH_3 symmetric stretching is calculated to be 2927 cm^{-1} and reported at 2935 cm^{-1} with medium intensity in the IR spectrum. High contributions of C=O stretching band is calculated to be at 1748 cm^{-1} and match nicely to the peak at 1719 cm^{-1} with weak intensity in the Raman and with strong intensity at 1718 cm^{-1} in the IR spectrum. This multiple bonded group is highly polar, therefore, gives rise to an intense IR band.

5. Conclusions

In this work, we have performed the experimental and theoretical vibrational and electrostatic potential surface analysis of the YHCl. These assignments are important to understand the molecular structure and biological activity of the title molecule. The equilibrium geometries and harmonic vibrational wavenumbers of all the 156 normal modes of the molecule were determined and analyzed both at DFT and HF levels of theory employing the 6-311++G(d,p) basis set. Information about the size, shape, charge density distribution and structure-activity relationship of the YHCl molecule has been obtained by mapping electron density isosurface with ESP. Raman and infrared spectra were recorded, and the vibrational bands were assigned on the basis of the PED obtained from the DFT calculations.

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