

Differential Scanning Calorimetry, NBO and Hyperpolarizability Analysis of Yohimbine Hydrochloride

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Abstract

Yohimbine ($C_{21}H_{27}N_2O_3$) is one of the most important indole alkaloid. Differential scanning calorimetry, natural bond orbital (NBO) analysis and dipole moment with hyperpolarizability have been performed for molecular characterization. Stability of the molecule arising from hyper conjugative interactions, charge delocalization has been analyzed using NBO analysis. The results show that charge in electron density (ED) in the σ^* and π^* anti bonding orbitals and $E(2)$ energies confirms the occurrence of intra molecular charge transfer (ICT) within the molecule.

Keywords: YHCl, DSC, NBO, Hyperpolarizability.

Introduction

Indole alkaloid group, the naturally-occurring heterocyclic amines derived from botanical sources, has received more chemical attention during the last decade. They have been recoized for their great medical value. Yohimbine, found in the stem bark of tall evergreen tree *Pausinystilia yohimbe*, which grows in tropical West Africa and Congo, also in the roots of *Rauwolfia serpentina*, is one of most reputed indole alkaloid (Ambadey *et al.*, 1973; van Tamelen *et al.*, 1969; Quinton, 1963). Among many other extracts, yohimbine hydrochloride (YHCl) is a Food and Drug Administration (FDA) approved prescription drug for the treatment of impotence (Betz *et al.*, 1995). It has been used since ancient time for its potent aphrodisiac property (Ambadey *et al.*, 1973; van Tamelen *et al.*, 1969; Quinton, 1963; Eweka *et al.*, 2010], and has been used even in the crude form by African natives. It is promoted for erectile dysfunction, weight loss and depression (Goldberg and Robertson, 1983; Tank *et al.*, 2007). Yohimbine has also been used in traditional medicine to treat high blood pressure, chest pain, age-related cognitive disorders and obesity (Yohimbine-Supplement Review, 2010). The optimized structure of YHCl is given in Fig. 1, below.

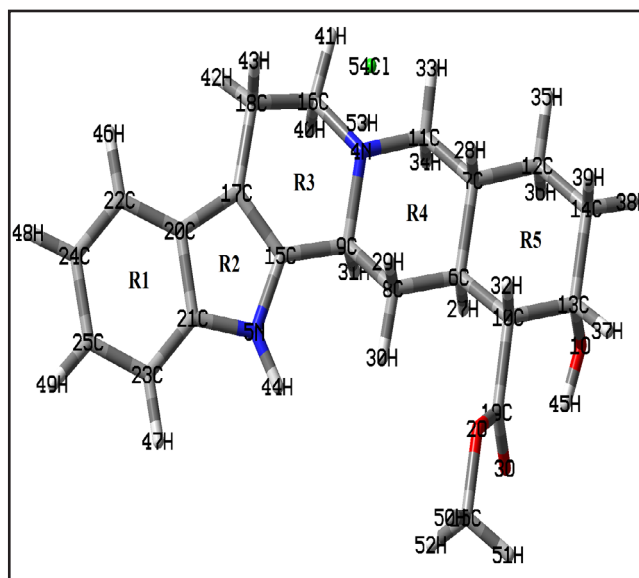


Fig. 1. Optimized structure of YHCl.

Recently, we have observed the experimental IR, Raman (noble tools of vibrational spectroscopy) and UV-vis spectra of the title molecule and analysed a comparison to their theoretical calculations with full potential energy distribution (PED) analysis (Joshi *et al.*, 2011). In this communication we have given natural bond orbital (NBO) analysis, static dipole moment, polarizability by density functional theory (DFT) and differential scanning calorimetry (DSC). NBO

analysis elucidates the charge delocalization within the molecule. DSC analysis, a thermal experimental technique, helps to know the purity as well as the transition temperatures (like glass transition, melting, boiling, decomposition etc). We have used DSC to find the melting point of YHCl.

Methodology

Experimental

The YHCl in powder form was extracted from *Rauwolfia serpentine* Benth. (Apocynaceae) by acid base extraction method and purified by a combination of column and preparative thin layer chromatography, converted to its hydrochloride, crystallized from methanol–ether, m. p. 299.5° [301°] (Warnat, 1930; Swan, 1950). The compound was identified as yohimbine by a direct comparison with an authentic sample procured from Sigma Chemical Company, USA.

Differential scanning calorimetry (DSC) analysis was performed using a Mettler Toledo 821° DSC (Mettler Toledo, Switzerland) operating with version 5.1 of Star° software. The sample (3.04 mg) was encapsulated in aluminium pan having pierced lids to allow escape of volatiles. The heating rates of 10 °C min⁻¹ and nitrogen purge at 30 ml min⁻¹ were employed. The temperature axis and the cell constant were calibrated using indium.

Computational

NBO analysis and the polarizability properties of the molecule were computed by the DFT method using the Gaussian 09 program (Frisch *et al.*, 2009) package at Becke's three parameter (local, nonlocal, Hartree–Fock) hybrid exchange functional with Lee–Yang–Parr correlation functional (B3LYP) (Lee *et al.*, 1998; Becke, 1993; Parr and Yang, 1989) employing 6-311++G(d,p) basis set. 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. All the donor and acceptor occupancies with their interaction energies were reported with the help of obtained second-order micro perturbation theory.

Results and discussions

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC), is a fundamental tool in thermal analysis, has been widely used as an experimental technique for the study of a variety of phase transformations (Vazquez *et al.*, 1996; Borrego and Gonzalez-Doncel, 1998; Vazquez, 1997). It can be used in many industries – from pharmaceuticals to polymers and from nanomaterials to food products. The information these instruments generate is used to understand amorphous and crystalline behavior, polymorph and eutectic transitions, curing and degree of cure, and many other material properties used to design, manufacture and test products.

DSC analysis showed an endothermic peak for YHCl. The melting endothermic during heating was occurred at 297.87 °C (onset-peak-endset) with enthalpy of 270.18 J/g, while during cooling an exothermic peak was occurred at 249.98 °C with enthalpy of 90.47 J/g (Fig. 2). From the curve it is clear that no glass transition has occurred so far.

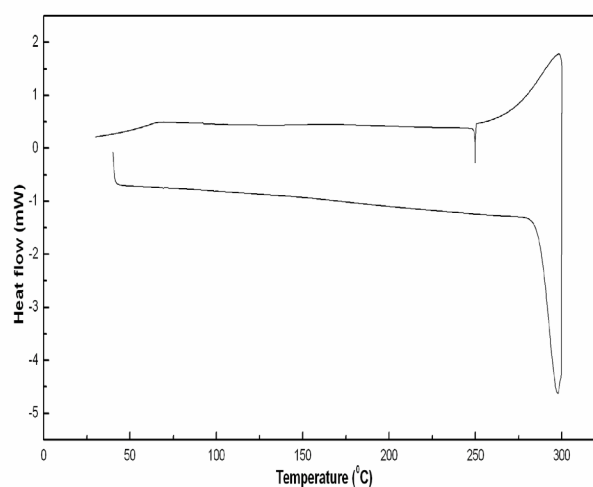


Fig. 2. DSC curve of YHCl for a heating rate 10 °C/min in N₂ atmosphere.

Natural Bond Orbital (NBO) analysis

NBO analysis is based on a method for electronic structure description like as the classic Lewis bonding theory (Weinhold and Landis, 2005, 2001; Reed *et al.*, 1988). It provides a convenient basis for investigating charge transfer or conjugative interaction in molecular systems, which enhance the analysis of intra- and

intermolecular charge interaction between the localized NBO and empty molecular bonds. Using the second-order micro disturbance theory, we have reported the occupancy of the filled NBOs (bonds, lone pairs) and the empty NBOs (antibonds, Rydbegs), as well as their interaction energies (James *et al.*, 2006; Na *et al.*, 2005). The electron delocalization from filled NBOs (donors) to the empty NBOs (acceptors) describes as a conjugative electron transfer process between them. Higher the stabilization energy E(2), more intense is the bond interaction. For each donor (*i*) and acceptor (*j*), the stabilization energy E(2) associated with the delocalization $i \rightarrow j$ is estimated as (Weinhold and Landis, 2005, 2001):

$$E(2) = -n_{\sigma} \frac{\langle \sigma | F | \sigma \rangle^2}{\varepsilon_{\sigma^*} - \varepsilon_{\sigma}} = -n_{\sigma} \frac{F_{ij}^2}{\Delta E}$$

where $(\langle \sigma | F | \sigma \rangle)^2 < \langle \sigma | F | \sigma \rangle^2$ or F_{ij}^2 F_{ij}^2 is the Fock matrix element which corresponds to *i* and *j* NBO orbitals. n_{σ} is the population of the donar σ orbital, ε_{σ^*} & ε_{σ} are the energies of σ^* & σ NBOs.

Lower occupancy (i.e., electron density) of bonding NBO means more electrons have been donated into antibonding NBOs (Bing *et al.*, 2008), thus lower occupancy leads to stronger donor-ability. From Table 1, it can be seen that the values of occupancy are 1.96053-1.99349 and 1.72711-1.98400 for σ and π bonding NBOs respectively. Therefore, π bonding NBOs possesses higher donor-ability than σ bonding NBOs. The π bonding NBO at C22 - C24 has lowest occupancy 1.72711, hence posses the strongest donor ability among all the bonding NBOs. Similarly, larger occupiencies of the antibonding NBOs leads to the stronger acceptor ability. As to the parameters of antibonding NBOs shown in Table 1, the occupancies of π^* NBOs are much larger than those of σ^* NBOs and hence have stronger acceptor ability.

Table 1. Selected second order perturbation theory analysis of Fock matrix in NBO basis of YHCl.

Donor NBO	ED (i)/e	Acceptor NBO	ED (j)/e	E(2)	E(j)-	F(i,j) ^c
				^a kJ mol ⁻¹	E(i) ^b a.u.	a.u.
LP(2) O1	1.96001	σ^* C13 - C14	0.03307	30.01	0.67	0.062
LP(1) O2	1.96438	σ^* O3 - C19	0.02195	33.57	1.12	0.085
LP(2) O2	1.78826	π^* O3 - C19	0.22692	220.70	0.31	0.115
		σ^* C26 - H51	0.01353	17.97	0.76	0.054
LP(2) O3	1.84742	σ^* C26 - H52	0.01386	18.22	0.76	0.054
		σ^* O2 - C19	0.10495	149.77	0.60	0.133
LP(1)Cl 54	1.99921	σ^* C10 - C19	0.06508	79.88	0.64	0.101
		σ^* N4 - H53	0.23404	33.86	1.02	0.086
LP(4)Cl 54	1.76221	σ^* N4 - H53	0.23404	377.37	0.56	0.201
σ N5 - C15	1.98211	σ^* C21 - C23	0.02205	17.31	1.39	0.068
		σ^* C 9 - C15	0.02798	18.27	1.22	0.065
σ C8 - H30	1.97396	σ^* N4 - C9	0.03944	20.65	0.75	0.054
σ C9 - C15	1.97443	σ^* C15 - C17	0.02234	18.35	1.29	0.067
σ C9 - H31	1.96971	π^* C15 - C17	0.31644	17.31	0.57	0.047
σ C10 - H32	1.95883	π^* O3 - C19	0.22692	18.73	0.49	0.044
σ C15 - C17	1.96638	σ^* C20 - C22	0.02352	19.35	1.29	0.069
σ C17 - C20	1.96477	σ^* C 9 - C15	0.02798	24.96	1.07	0.071
		σ^* C20 - C22	0.02352	19.45	1.23	0.068
σ C18 - H42	1.97136	σ^* N4 - C16	0.02942	19.86	0.75	0.053
σ C18 - H43	1.97121	π^* C15 - C17	0.31644	18.60	0.53	0.047
σ C20 - C21	1.96053	σ^* C17 - C18	0.02256	21.03	1.09	0.066
		σ^* C21 - C23	0.02205	18.56	1.24	0.067
σ C20 - C22	1.97389	σ^* C17 - C20	0.02869	21.74	1.23	0.071
		σ^* C20 - C21	0.02807	16.80	1.23	0.063
σ C21 - C23	1.97560	σ^* C20 - C21	0.02807	19.81	1.25	0.069
σ C22 - C24	1.97856	σ^* C17 - C20	0.02869	19.90	1.23	0.068
σ C22 - H46	1.98132	σ^* C22 - C24	0.01453	17.56	1.06	0.060
σ C23 - C25	1.97578	σ^* N5 - C21	0.02478	26.50	1.13	0.076
σ C23 - H47	1.98103	σ^* C20 - C21	0.02807	17.47	1.07	0.060
σ O2 - C19	1.99349	σ^* C6 - C10	0.02477	19.98	0.03	0.043
		π^* C15 - C17	0.31644	74.74	0.08	0.044
σ N5 - C21	1.98400	π^* C23 - C25	0.01461	365.79	0.07	0.096
		π^* C15 - C17	0.31644	89.03	0.37	0.084
π N5 - C21	1.98400	π^* C23 - C25	0.33007	22.70	0.37	0.042
		π^* C15 - C17	0.79516	27.84	0.23	0.042
π C15 - C17	1.96638	π^* C23 - C25	0.33007	84.10	0.28	0.067
π C22 - C24	1.72711	π^* C5 - C21	0.79516	145.13	0.21	0.088
π C23 - C25	1.73199	π^* C22 - C24	0.30730	73.69	0.29	0.064

^a E(2) means energy of hyper conjugative interaction (stabilization energy). ^bEnergy difference between donor and acceptor *i* and *j* NBO orbitals. ^cF(*i*, *j*) is the Fock matrix element between *i* and *j* NBO orbit als.

The lone pair (LP) to the anti-bonding acceptor interactions, related to the resonance in the molecule, are electron delocalization from O2 atom as LP(2) \rightarrow π^* (O3 - C19) of carbonyl group and LP(2) \rightarrow σ^* (C26 - H51)/LP(2) \rightarrow σ^* (C26 - H52) of the methyl group. This shows the conjugation between the electron donation group and the acceptors, which correspond

to the stabilization energy 220.70 and ~18 kJ mol⁻¹, respectively. While the lone pair electron interaction between C154 atom and the antibonding σ*(N4-H53) of the ring R3, which leads to the stabilization energy 377.37 kJ mol⁻¹. σ(N5 - C21) bonding electron conjugated with π*(C15 - C17) and π*(C23 - C25) result stabilization energy 74.74 and 365.79 kJ mol⁻¹, respectively. The electron delocalization from π(C23 - C25) to antibonding π*(C5 - C21) and π*(C22 - C24) in the ring R1 leads to the stabilization energy 145.13 and 73.69 kJ mol⁻¹, respectively. These various interactions between the filled NBOs and empty NBOs show the intramolecular charge transfer (ICT) in the molecule.

Dipole Moment, Mean Polarizability and First Hyperpolarisability

The first hyperpolarizability (β₀) and related properties (β, α₀ and Δα) of this novel molecular system, YHCl were calculated, based on the finite-field approach, following the B3LPY level of theory employing 6-311++G(d,p) basis set. In the presence of an applied electric field, the energy of a system is a function of the electric field also. First hyperpolarizability is a third rank tensor of order three that can be described by a 3x3x3 matrix. The 27 components of the 3D matrix can be reduced to 10 components using the Kleinman Symmetry (Kleinman, 1962) and it can be given in the lower tetrahedral format. It is obvious that the lower part of the 3x3x3 matrix is a tetrahedral. The components of β are defined as the coefficients in a Taylor series expansion of the energy in the external electric field. When the external electric field is weak and homogeneous, the expansion of the energy in the external electric field can be written as:

$$E = E^0 - \mu_\alpha F_\alpha - 1/2 \alpha_{\alpha\beta} F_\alpha F_\beta - 1/6 \beta_{\alpha\beta\gamma} F_\alpha F_\beta F_\gamma + \dots$$

where E⁰ is the energy of the unperturbed molecules, F_α is the field at the origin and μ_α, α_{αβ}, and β_{αβγ} are the components of dipole moment, polarizabilities, and the first hyperpolarizabilities, respectively. The polarizability α and the hyperpolarizability β are the second and third rank tensors, respectively (Karna *et al.*, 1991). The total static dipole moment μ, the mean polarizability α₀, the anisotropy of the polarizability Δα, and the first hyperpolarizability β₀ are defined as

follows using the x, y, z components:

$$\mu = (\mu_x^2 + \mu_y^2 + \mu_z^2)^{1/2}$$

$$\alpha_0 = 1/3(\alpha_{xx} + \alpha_{yy} + \alpha_{zz})$$

$$\Delta\alpha = 2^{-1/2}[(\alpha_{xx} - \alpha_{yy})^2 + (\alpha_{yy} - \alpha_{zz})^2 + (\alpha_{zz} - \alpha_{xx})^2 + 6\alpha_{xx}^2]^{1/2}$$

$$\beta_0 = (\beta_x^2 + \beta_y^2 + \beta_z^2)^{1/2}$$

Table 2: Total static dipole moment μ (Debye), mean polarizability α₀ (x10⁻²⁴esu), anisotropy of polarizability Δα (x10⁻²⁴esu), and the mean first hyperpolarizability β₀ (x10⁻³⁰esu) of YHCl.

Total static dipole moment μ (Debye)	μ _x = -4.7996 μ _y = 2.7117 μ _z = -6.4919 μ = 8.5167
Mean polarizability α ₀ (x10 ⁻²⁴ esu)	α _{xx} = -140.881 α _{xy} = 0.2225 α _{yy} = -161.7081 α _{xz} = -7.5197 α _{yz} = 26.7223 α _{zz} = -189.0896 α ₀ = -163.8927x10 ⁻²⁴
Anisotropy of polarizability Δα (x10 ⁻²⁴ esu)	Δα = 247.45229x10 ⁻²⁴
Mean first hyperpolarizability β ₀ (x10 ⁻³⁰ esu)	β _{xxx} = -195.1511 β _{xyy} = -38.5857 β _{xzz} = -50.2414 β _{yyy} = 107.9132 β _{xyx} = 18.6593 β _{yyz} = 41.4066 β _{zzz} = -52.4277 β _{xxz} = 11.3243 β _{yyz} = -40.8924 β _{xyz} = -0.5263 β ₀ = 1692.4090x10 ⁻³⁰

$$\text{And, } \beta_x = \beta_{xxx} + \beta_{xyy} + \beta_{xzz}$$

$$\beta_y = \beta_{yyy} + \beta_{xyx} + \beta_{yzz}$$

$$\beta_z = \beta_{zzz} + \beta_{xxz} + \beta_{yyz}$$

The calculated values of the total static dipole moment μ, the mean polarizability α₀, the anisotropy of the polarizability Δα, and the mean first hyperpolarizability β₀ of YHCl are given in Table 2.

Conclusion

The title molecule has a sharp melting point at 297.87 °C, but no any glass transition has been observed. No

polymorphic change was observed within the melting. Based on a combined study of DFT with NBO method, a detailed bonding analysis on the title molecule is performed. NBO analysis result reflects the charge transfer within the molecule. Mostly the antibonding π NBOs of the indole part possess higher acceptor-ability, while the bonding π NBOs have stronger donor-ability.

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