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Spectroscopic and theoretical study of the *o*-vanillin hydrazone of the mycobactericidal drug isoniazid

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ABSTRACT

A complete and detailed study of the hydrazone obtained from condensation of antituberculous isoniazid (hydrazide of the isonicotinic acid, INH) and *o*-vanillin (2-hydroxy-3-methoxybenzaldehyde, *o*-HVa) is performed. It includes structural and spectroscopic analyses, comparing experimental and theoretical results. The compound was obtained as a chloride of the pyridinic salt ($INHOVA^{+}Cl^{-}$) but it will be referred as INHOVA for the sake of simplicity. The conformational space was searched and optimized geometries were determined both in gas phase and including solvent effects. Vibrational (IR and Raman), electronic and NMR spectra were registered and assigned with the help of computational methods based on the Density Functional Theory. Isoniazid hydrazones are good candidates for therapeutic agents against tuberculosis with conserved efficiency and lower toxicity and resistance than parent INH.

1. Introduction

Isoniazid, the hydrazide of isonicotinic acid (INH, see Scheme 1) has been recognized as an effective antituberculous agent since the middle of the 20th century. It is still employed in the treatment and prevention of this disease, not only as a single drug but also combined with others. Unfortunately, the actual formulations show several undesired collateral effects and can even cause irreversible damage in the liver in chronic patients. This fact, together with the resistance that microorganisms develop against these drugs, encouraged the search for new compounds with therapeutic effects [1,2]. The combination of INH with some hydroxyaldehydes leads to the formation of stable hydrazones that show conserved activity and less toxicity, due to the inactivation of the NH_2 group of INH [3]. Particularly, a group of hydrazones has been determined to be more effective than INH itself as antituberculous agents in macrophages [4]. This result is important, since the goal of a therapy against tuberculosis is to eradicate the *Mycobacterium* both in the extracellular and intracellular environment.

Among the hydroxyaldehydes that can react with INH, vanillin (4-hydroxy-3-methoxybenzaldehyde, HVa, see Scheme 2) has been used for analytical determination of INH in blood by formation of a colored compound (phtivazide or vanicide) [5]. This colorimetric method is still used nowadays for quantification of INH in pharmaceutical formulations [6]. Both HVa and its isomer *o*-HVa (2-hydroxy-3-methoxybenzaldehyde, see Scheme 2) have antioxidant activity, due to their ability to capture free radicals. This behavior is related to the presence of the OH moiety in their structure [7]. The low toxicity and therapeutic properties of these aldehydes make them good candidates for the preparation of hydrazones with a better pharmacological profile. Taking into account that tuberculosis, as other endemic diseases caused by parasites, mainly affects population of low economical level, the development of low-cost drugs is also an important objective in the research field [8].

It is well known that the biological activity of a compound is strongly dependent on its physicochemical, structural and electronic properties. Moreover, INHOVA could be a suitable ligand for the development of coordination compounds because of its potential ability to coordinate several metal ions of biological and pharmacological interest through its N and O donor atoms [9,10]. Thus, the detailed physicochemical characterization of organic compounds that can be used in the development of therapeutic agents is an important tool in the understanding of its mechanism of interaction both with biological substrates as with biometallic centers. Additionally, complementary studies on the solution chemistry of such a drug candidate, even when performed in an

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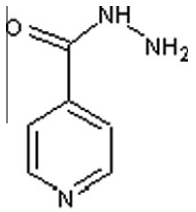
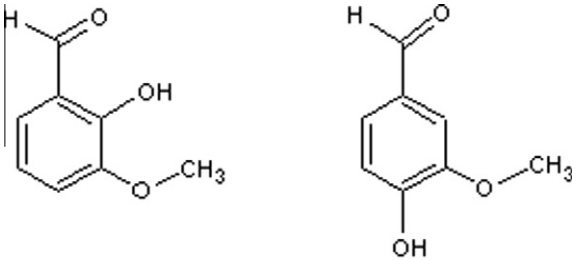
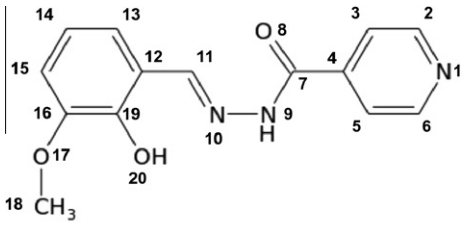


INH

Scheme 1. Schematic representation of isoniazid (INH).



Scheme 2. Schematic representation of vanillin (HVa) and its isomer *o*-vanillin (*o*-HVa).



INHOVA

Scheme 3. Schematic representation of the hydrazone obtained from condensation of INH and *o*-HVa (INHOVA$^+$).

organic solvent, could shed light on the system and give us some information about the nature of the species occurring in INHOVA solutions.

In this work we report a detailed study of the hydrazone obtained from condensation of INH and *o*-HVa in methanol, differing from the procedure described in the literature for its synthesis [11]. The compound was obtained as a chloride of the pyridinic salt (INHOVA$^+$Cl$^-$) but it will be referred as INHOVA (see Scheme 3) for the sake of simplicity. The work includes experimental and theoretical results. Vibrational (IR and Raman), electronic and NMR spectra were registered and assigned with the help of computational methods based on the Density Functional Theory.

2. Experimental methods

2.1. Synthesis

All chemicals were of analytical grade and were used as purchased. The compound was prepared according to the reported procedure for the reaction of HVa and INH employed as analytical method [6], replacing ethanol by methanol and HVa by *o*-HVa. Ten milliliter of methanolic solution containing 3 mmol of *o*-HVa (Sigma) was drop-wise added to a solution of 1.5 mmol of INH (Fluka) in 16 mL of methanol (Merck), with continuous stirring and slight heating. Drops of concentrated hydrochloric acid (Merck) were added to reach a pH value of 5. The starting pale yellow solution turned immediately to bright yellow and a precipitated was formed. The system was left in digestion for 2 h and the solid was filtered and washed with methanol. This procedure differs from the previously reported by Chen et al. [11]. The product corresponds to the hydrazone hydrochloride hemi-hydrate: INHOVA·HCl·½H_2O (Yield: 78%; m.p: 228–229 °C; Anal. Calcd. for $C_{14}H_{15}O_{3.5}N_3Cl$: C, 53.04%; H, 4.74%; N, 13.26%. Found: C, 52.24%; H, 4.59%; N, 12.98%).

2.2. Spectroscopic analysis

IR spectra were recorded with a Bruker 113v FTIR instrument, using the KBr pellet technique. Raman spectra were obtained with a Perkin–Elmer Raman Station 400, using the 785 nm line for excitation. Carbon, hydrogen and nitrogen determinations were performed in an elemental analyzer EA 1110 from CE Instruments. Melting point was determined with a MQAPF-302 Micro-Química instrument. The electronic absorption spectrum of the compound (5×10^{-5} M) was measured on methanolic solution in the 200–800 nm spectral range. It was recorded with a Hewlett–Packard 8452-A diode array spectrometer, using 10 mm quartz cells. ^{1}H and ^{13}C NMR spectra were obtained at room temperature in a Varian Mercury 200 spectrometer (4.7 T, 200 MHz for the ^{1}H nuclei) using a 5 mm probe and d_6-DMSO as solvent. Calibration was made with the solvent residual peaks as references (2.50 and 39.52 ppm for hydrogen and carbon, respectively) [12].

2.3. Computational methods

The conformational space of the protonated form of INHOVA was investigated with the aid of the semiempirical PM3 method [13]. Several starting geometries derived from selected variations in the C5—C4—C7—N9, C4—C7—N9—N10 and N9—N10—C11—C12 dihedral angles were considered to investigate how rings orientate relative to each other, see Scheme 3 for atom labeling. Moreover, dihedral angles involving the OH and OCH_3 groups were also accounted for to achieve an adequate description of the conformational space of the molecule under study.

Geometries were further optimized using the hybrid meta-GGA M06–2X exchange–correlation density functional [14] with a triple-zeta 6–311+G(d,p) basis set [15,16]. Numerical integrations were carried out using a grid containing 96 radial points and 590 angular points around each atom. The critical points found after optimization were characterized by the sign of the eigenvalues of the Hessian matrix of the total electronic energy with respect to the nuclear coordinates. When the critical point corresponded to a minimum on the potential energy surface of the molecule, the eigenvalues were converted to harmonic vibrational frequencies and a set of thermodynamic functions were calculated. Vibrational frequencies were scaled by a factor of 0.982 to ease the comparison with experimental values [14].

Solvent effects (methanol) were included implicitly through the Conductor-like Polarizable Continuum Model [17,18] to obtain the free energy of solvation. Geometries were kept frozen to their gas-phase structures, thus only wavefunction polarization effects were taken into account.

Electronic transitions were calculated within the framework of the Time-Dependent Density Functional Theory [19,20]. In this case, a coarser grid was used for numerical integrations containing 96 radial points and 302 angular points around each atom.

Geometry optimizations, Hessian matrix calculation and diagonalization, and electronic transition calculations were performed with the GAMESS-US program [21].

Isotropic magnetic shieldings of ^{13}C and ^{1}H were calculated at the B3LYP/6–311+G(2d,p)//B3LYP/6–31G* level of theory as