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FT-IR and FT-Raman spectra of 5-fluoroorotic acid with solid state simulation by DFT methods

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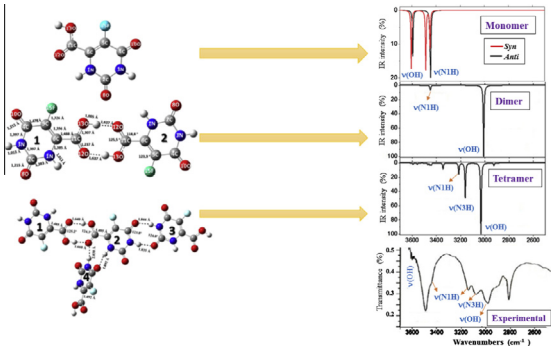
HIGHLIGHTS

- All the tautomers of 5-fluoroorotic acid were described and analyzed.
- The Raman and IR spectra of 5-fluoroorotic acid in the solid state were simulated.
- The solid state simulation was carried out by a dimer and tetramer forms.
- The characteristic skeletal modes in the low wavenumber range were determined.
- The solid state scaled values are in good accordance to the experimental ones.

GRAPHICAL ABSTRACT

Solid state simulation of 5-fluoroorotic acid.



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ABSTRACT

FT-Raman and FT-IR studies of the biomolecule 5-fluoroorotic acid in the solid state were carried out. The unit cell found in the crystal was simulated as a tetramer form by density functional calculations. They were performed to clarify wavenumber assignments of the experimental observed bands in the spectra. Correlations with the molecule of uracil were made, and specific scale equations were employed to scale the wavenumbers of 5-fluoroorotic acid. Good reproduction of the experimental wavenumbers is obtained and the % error is very small in the majority of the bands. This fact confirms our simplified solid state model. The molecular structure was fully optimized using DFT and MP2 methods. The relative stability of both the *syn* and *anti* conformations was investigated, and the *anti*-form was found to be slightly more stable, by 7.49 kJ/mol at the MP2 level. The structures of all possible tautomeric forms were determined. The *keto*-form appeared as the most stable one. The NBO atomic charges and several thermodynamic parameters were also calculated.

Introduction

Pyrimidines are single ring structures, and the pyrimidine bases are all derivatives of the parent compound pyrimidine. Its derivatives constitute a very important class of compounds because they

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are components of the biologically important nucleic acids and have been shown to exert profound physiological effects, and they have been used as antitumor, antibacterial, and antiviral drugs. The orotic acid (uracil-6-carboxylic acid, OA) or vitamin B_{13} and its salts play an important role in biological systems as precursors of pyrimidine nucleoside and are found in cells and body fluids of many living organisms [1a]. 5-Nitroorotic acid, a nitro-derivative of naturally occurring orotic acid (vitamin B_{13}), is a key intermediate in the biosynthesis of the pyrimidine nucleotides of DNA and RNA [1b–d]. These compounds are used in medicine as biostimulators of the ionic exchange processes in organisms. There is also a great interest in the study of orotic acid in relation to food protection and nourishment research [1a]. Orotic acid and its complexes with metal ions have attracted growing attention in medicine: they are used in prophylaxis and treatment of heart diseases and in curing syndromes associated with a deficiency of the essential metal ions [1e].

5-Fluoroorotic acid (5-fluorouracil-6-carboxylic acid, 5-FOA) is a synthetic fluoro derivative of naturally occurring orotic acid (OA). It is a precursor in the novo biosynthesis of series of purines and pyrimidines which has been used in the treatment of certain malignant conditions [2–5]. It induces chromosome alterations [2], and it inhibits the synthesis of mature cytoplasm ribosomal RNA in rat liver cells. It is a noncompetitive inhibitor of dihydroorotase, and it shows antitumor activity against transplanted tumors in rats and mices. It is also a potent inhibitor for some metalloproteins such as dihydroorotase and dihydroorotase dehydrogenase and for thymidylate synthase (nonmetalloprotein) in the human malaria parasite *Plasmodium falciparum* [3,6]. It is incorporated into the ribonucleic acid of animal cells *in vivo,* presumably after decarboxylation to fluorouracil. It has been used too as a selective toxic to yeast cells which synthesize orotidine-5′-phosphate decarboxylase (OMPdecase).

5-FOA is a bactericidal pyrimidine analogue. When it is converted into 5-fluorouridine monophosphate (5-FUMP) it can be incorporated into RNA, as well as further metabolized to 5-fluoro-2′-desoxiuridine monophosphate (5-FdUMP) to act as a potent inhibitor of thymidylate synthase, causing cessation of DNA synthesis. The key enzymes of this pathway are orotate phosphoribosyl transferase (OPRTase) (encoded by pyrE) and orotidine 5′-monophosphate decarboxylase (OMPdecase) (encoded by pyrF). Mutants that lack either of these enzymes are unable to metabolize 5-FOA and hence are resistant to it [7]. 5-FOA can also be considered as a prodrug of 5-fluorouracil (5-FU), which is a potent anticancer and antitumoral drug [3], and has been used against several types of cells [8].

Considering the importance of 5-FOA for medicinal chemistry, however, theoretical studies from the spectroscopy point of view are scarce and in general referred to the calculation of several properties. Also, their vibrational spectra have been relatively little looked into and it has not been completely and rigorously studied yet. Therefore, this is the task undertaken in the present work and as well to make the assignments to different normal modes following the notations of normal modes of the uracil molecule. We have previously analyzed the IR and Raman spectra of 5-aminoorotic acid [9] and several other uracil derivatives [10–12].

Computational methods

The calculations were carried out using MP2 *ab initio* method and Density Functional methods (DFT) [13], including with the Becke's three-parameter exchange functional (B3) [14] in combination with both the correlational functional of Lee, Yang and Parr (LYP) [15]. The B3LYP represents the most cost-effective method [16–19] and therefore it was the only one used in the present manuscript. The B3LYP method was also chosen because different studies have shown that the data obtained with this level of theory were in good agreement than those obtained by other more computational costly methods, such as MP2, and it predicts vibrational wavenumbers of DNA bases better than the HF and MP2 methods [20a], and moreover, it gives the lowest errors in uracil and its derivatives [20b]. All the methods used appear implemented in the GAUSSIAN 03 program package [21]. The UNIX version with standard parameters of this package was running in the alpha computer of the University Complutense of Madrid, Spain.

Several basis sets were utilized starting from the 6-31G(d,p) to 6-311++G(3df,pd). It was noted that 6-31G(d,p) leads to results that represent a compromise between accuracy and computational cost, and for this reason it was the main basis set used in the calculations. The 6-311++G(3df,pd) was utilized for the optimization because it appears close to the energy convergence limit. These basis sets have been previously tested on the uracil molecule [20,22].

The optimum geometry was determined by minimizing the energy with respect to all geometrical parameters without imposing molecular symmetry constraints. Berny optimization under the TIGHT convergence criterion was used. The keyword FREQ was employed for the wavenumber calculations in the harmonic approximation, with the RAMAN option for Raman values. No imaginary wavenumbers appear in the DFT calculated spectra. The natural NBO atomic charges [23] are one of the most accurate today to correlate properties, and they were determined with the keyword POP = NPA.

Experimental

The pure sample of 5-fluoroorotic acid of spectral grade (solid powder) was purchased from Aldrich Chemical Co, USA and used as such without any further purification for recording the FT-IR and FT-Raman spectra. The mid infrared spectrum of the compound in the in the region 400–4000 cm^{-1} was recorded with a Bruker IFS-66 Fourier transform spectrometer equipped with a Globar source, Ge/KBr beamsplitter and a TGS detector at room temperature. For the spectrum acquisition, 50 interferograms were collected at 4 cm^{-1} resolution.

The FT-Raman spectrum in the region 50–4000 cm^{-1} was recorded on a Bruker IFS-66 optical bench with a FRA 106 Raman module attachment interfaced to a microcomputer. The sample was mounted in the sample illuminator using optical mount and no presample pretreatment of any kind was undertaken. The NIR output (1064 nm) of an Nd:YAG laser was used to excite the spectrum. The laser power was set at 250 mW and the spectrum was recorded over 500 scans at a fixed temperature. The spectral resolution was 6.0 cm^{-1} after apodisation.

The full spectra are shown in supplementary material section.

Results and discussion

Geometry optimization in the isolated state

Two possible locations for the carboxylic proton appear in 5-FOA, Fig. 1, according to the value of N1—C6—C11—O13 dihedral angle 0.0° or 180.0°. Thus, two stable conformers were found, designated according to the notation used in OA [1a] as 1 (*syn*) and 2 (*anti*), respectively, Fig. 1. However, we have changed the definition of θ to be the angle N1—C6—C11—O13 ($\theta = 0°$, *syn*) instead of N1—C6—C11═O12 ($\theta = 180°$, *syn*) of Ref. [1a]. The most stable one, conformer 2 is stabilized by a stronger intramolecular interaction through H7 than in conformer 1. This *anti* orientation of the carboxylic group is in accordance to the X-ray data of OA [24].