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Determination of preferred conformations of ibuprofen in chloroform by 2D NOE spectroscopy

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ABSTRACT

Solution of an anti-inflammatory drug ibuprofen ((RS)-2-(4-isobutylphenyl) propionic acid) in chloroform was studied by nuclear magnetic resonance spectroscopy. A set of 2D NOESY spectra was analyzed in order to obtain atom–atom distances. Since ibuprofen is known to exist as an ensemble of different conformations, these distances are averaged over the ensemble. To compare experimental and calculated distances, three models of averaging were concerned. Our data allowed to determine the dominant conformers of ibuprofen dissolved in chloroform. The population of conformers in the saturated solution leads to a certain crystal morphology formed within the nucleation process. Observed and calculated ^{13}C chemical shifts (at the DFT/B3LYP/6-311+G(2d,p) level) were in good agreement.

1. Introduction

Information on properties of conformations of biologically active molecules, including nonsteroidal anti-inflammatory drugs, is of paramount importance for better understanding of the structure–activity relationships underlying their biological effect and of the mechanism of their action on an organism (Llorens et al., 2002; Marot et al., 2000; Selinsky et al., 2001). Experimental determination of spatial structure and conformational state of biologically active molecules attracts an increasing interest (Butts et al., 2012; Efimov et al., 2013; Fernandes et al., 2003; Khodov et al., 2013).

Ibuprofen ((RS)-2-(4-isobutylphenyl) propionic acid, $C_{13}H_{18}O_2$) is a nonsteroidal anti-inflammatory drug used in treating rheumatoid arthritis, osteoarthritis, and other diseases for pain relief and alleviation of fever (Adams et al., 1967). It was firstly synthesized by Adams with his colleagues in 1961 and called BTS 13621. It has an outstanding biological activity among substituted phenylalkane and alkene acids (Adams, 1992; Adams et al., 1967).

The ibuprofen molecule can be regarded as a benzene ring having two para-substituents (Fig. 1). One of them is the $–CH_2–CH–(CH_3)_2$ chain, and the other contains a carboxyl group ($–CH(CH_3)COOH$). Ibuprofen molecules possess a chiral centre at the α-carbon atoms (C6 in Fig. 1) and can exist as R(−) and S(+) enantiomers. Commercially available ibuprofen is also a racemic mixture of both enantiomers. Geisslinger et al. have shown that only the S(+) form is pharmaceutically active (Geisslinger et al., 1989). The inactive R(−) ibuprofen, however, may undergo a unidirectional chiral inversion into the active S(+) form in vivo (Geisslinger et al., 1989; Lin et al., 2004).

Ibuprofen molecule is flexible due to internal rotations of the propionic acid fragment and the isobutyl group. Namely, it is determined by varying four dihedral angles around the C1–C6, C6–C3, C2–C7, and C7–C8 bonds: $\tau 1$ (O–C1–C6–C3), $\tau 2$ (C1–C6–C3–C4), $\tau 3$ (C5–C2–C7–C8), and $\tau 4$ (C2–C7–C8–C9), respectively. If the ibuprofen molecule is regarded as a para-substituted aromatic ring, its different forms can be described in terms of relative orientations of the substituents (below or above the ring plane). The rotations around the C6–C3 and C2–C7 bonds are not correlated, which is evidenced by comparing conformers pairwise (see Table 1). Variety of conformations results in variety of geometric and electronic properties of molecules in solution.

Eight possible different conformations of ibuprofen were found in Vueba et al. (2008) based on quantum chemical calculations. Having compared the results of vibrational spectroscopy and quantum chemical calculations, the authors suggest that a limited number of conformers can be considered due to a very small energy difference in pairs between the A and B, C and D, E and F, and G

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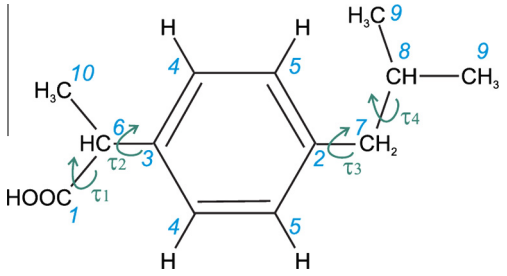

Fig. 1. Scheme of the ibuprofen molecule with atom numbering and dihedral angles responsible for the formation of different conformers.

and H conformations. The Boltzmann distribution of conformers in vacuum at the room temperature is as follows: 75.0% (A and B), 14.0% (C and D), 9.0% (E and F), and 2.0% (G and H). It is shown in Vueba et al. (2008) that the vibrational spectroscopy data reflect the presence of the A form only in the solid phase, most probably due to a better packing of this structure in the crystal. However, that work considered the sole solid crystalline phase I.

Based on DSC and X-ray data on racemates of ibuprofen, the second crystalline phase II (melting point 290 K) was revealed in addition to the already known crystalline phase I (melting point 349 K) (Dudognon and Danède, 2008). Its melting point is lower than that of the phase I and the Rietveld factors are high (Derollez and Dudognon, 2010). These observations, as well as Raman spectroscopy data (Hédoux et al., 2011), give a convincing evidence for the second crystalline phase being thermodynamically less stable than the phase I. Both of them belong to the monoclinic $P2_1/c$ space group but differ in the arrangement of molecules, as shown in Fig. 2 (Shankland et al., 1998).

It was shown in Mattei and Li (2012) that the conformers' molecular structure and interactions between dissolved drug molecules determine pre-nucleation and nucleation processes. Information on distribution of conformers in a saturated solution might facilitate understanding of the mechanism of formation of one or another crystalline phase. However, in spite of the fact that ibuprofen has been thoroughly studied, information of this kind is absent in the literature. Presence of multiple conformations in fast mutual exchange issues a serious challenge to researchers and requires developing of new ways of analyzing experimental data.

In this work, we determined preferred spatial structure and parameters of conformational equilibrium of ibuprofen in chloroform by two independent methods: nuclear Overhauser effect spectroscopy (NOESY) and comparison of NMR data (^{13}C chemical shifts) with quantum chemical calculations. A similar approach was used earlier to analyze a system undergoing two-site chemical exchange (Butts et al., 2012); here we expanded this approach to the case of a multi-conformer molecule. The choice of solvent

Table 1
Dihedral angles determining differences between the ibuprofen conformers based on the quantum-chemical calculated structures (Vueba et al., 2008). Where equivalent atoms have the same name (C4, C5, and C9), one of them was chosen for analysis in all structures.

Conformer	Dihedral angle (°)			
	τ1	τ2	τ3	τ4
A	88.9	54.8	105.1	172.4
B	89.0	−126.5	105.6	172.4
C	89.5	55.1	90.0	−63.0
D	89.4	−127.4	89.7	−63.6
E	−80.3	−114.5	103.7	172.2
F	−81.2	63.9	103.2	172.0
G	−81.6	−117.4	89.9	−63.2
H	−81.3	64.0	90.2	−62.5

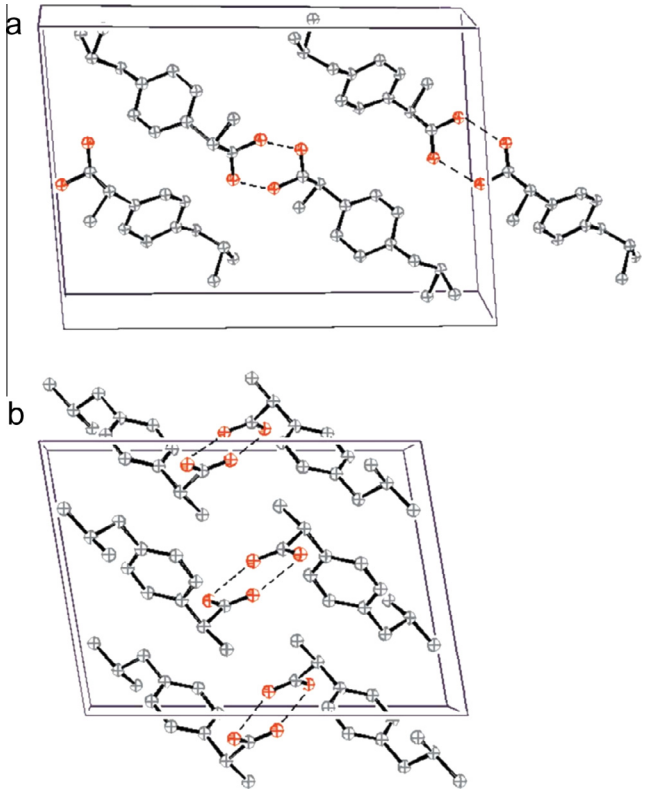

Fig. 2. Unit cells of two ibuprofen conformers: (a) phase I (Shankland et al., 1998) and (b) phase II (Derollez and Dudognon, 2010).

was justified by high solubility of ibuprofen in $CHCl_3$ and the practical significance of this solvent in the recrystallization process. Information on the distribution of conformers at maximal solution saturation may be used in studying processes of crystal nucleation from the solvent. Results of our experiments were also analyzed in the light of literature data, obtained by other methods.

2. Experimental and calculation details

2.1. NMR spectroscopy

Samples were prepared in 5 mm NMR tubes and contained typically 0.6 mL $CDCl_3$. Preparation was carried out under air without degassing. All NMR experiments were performed on a Bruker Avance III 500 NMR spectrometer equipped with a 5 mm probe using standard Bruker TopSpin Software. Temperature control was achieved using a Bruker variable temperature unit (BVT-2000) in combination with a Bruker cooling unit (BCU-05) to provide chilled air. Experiments were run at 298 K without sample spinning.

^1H NMR (500 MHz) spectra were recorded using 90° pulses and relaxation delay of 1 s; spectral width was 14 ppm; 128 scans were acquired. ^{13}C NMR spectra were recorded using 45° pulses, broadband decoupling from protons and relaxation delay of 2 s; spectral width was 200 ppm; 200 scans were acquired. NMR spectra were referenced relative to solvent peaks.

Two-dimensional Total Correlation Spectroscopy (2D ge-TOCSY) (Bax and Davis, 1985) experiments were performed with pulsed filtered gradient techniques. The spectra were recorded in a phase-sensitive mode using Echo/Antiecho-TPPI gradient selection with 2048 points in the F2 direction and 256 points in the F1 direction. Spin-lock delay values for 2D ge-TOCSY were