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Assessing the stable conformations of ibuprofen in solution by means of Residual Dipolar Couplings

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ABSTRACT

Detailing the conformational equilibria between global and local minimum energy structures of anti-inflammatory α-arylpropionic acids directly in solution is of the utmost importance for a better understanding of the structure-activity relationships, hence providing valuable clues for rational structure-based drug design studies. Here the conformational preferences of the widely used pharmaceutical ibuprofen were investigated in solution by NMR spectroscopy in weakly ordering phases. A thorough theoretical treatment of the anisotropic interactions that are relevant for NMR spectra led to a conformational model characterized by six pairs of symmetry-related conformers, in particular four couples of *gauche* structures, with a total probability of 93%, and 2 couples of *trans* structures, counting for the remaining 7%.

1. Introduction

Ibuprofen (2-(4-(2-methylpropyl)phenyl)propanoic acid) is a poorly water-soluble, widespread non-prescriptive drug, frequently used for the treatment of painful and inflammatory conditions, such as rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. It is listed by the World Health Organisation as one of the essential drugs for a basic health-care system (WHO, 2015).

Structurally, ibuprofen represents the parent compound of the 2-arylpropionic-type non-steroidal anti-inflammatory drugs (NSAIDs) commonly called “profens”, which inhibit the enzyme cyclooxygenase (COX) by interacting with its active site and thus competitively blocking it (Dannhardt and Kiefer, 2001; Hart and Huskisson, 1984).

Although it was first synthetized in the '60s (Adams et al., 1967) and its three-dimensional crystal structure was reported earlier (Freer, 1993; McConnell, 1974; Shankland et al., 1996, 1997), there is currently a renewed interest in the investigation of the conformational space of ibuprofen (Betz et al., 2015; Fu et al., 2011; Khodov et al., 2014a; Klimovich and Mobley, 2010; Liu and Gao, 2012; Oparin et al., 2016; Paluch et al., 2011; Vueba et al., 2008). This can be related to the growing awareness of the key role that the 3D structure plays in driving the biological activity of pharmaceutical compounds. Indeed, it is now clear that a deeper knowledge of the conformational features of biologically active molecules, including NSAIDs, can help in understanding why and how they work, shedding more light on the structure-activity relationships underlying their biological effects (Llorens et al., 2002; Selinsky et al., 2002; Smeyers et al., 1985). In addition, the conformational preferences affect the drug's transport property (Jämbeck and Lyubartsev, 2013; Loverde, 2014) and its release from delivery systems (Geppi et al., 2005) and hence its bioavailability. However, the experimental determination of spatial structure and conformational state of pharmaceuticals is still a serious challenge to researchers, especially in the presence of interconverting multiple conformations.

Ibuprofen consists of a benzene ring bridging two *para*-substituents, an isobutyl and a propanoic acid group (Fig. 1), so that a rich conformational landscape can be expected. The conformational problem for ibuprofen was addressed by several quantum-chemical calculations (Fu et al., 2011; Jubert et al., 2006; Klimovich and Mobley, 2010; Liu and Gao, 2012; Okulik and Jubert, 2006; Oparin et al., 2016; Paluch et al., 2011; Shankland et al., 1998; Villa et al., 2001, 2004; Vueba et al., 2008), predicting a large number of low-energy conformers. As alternative to theoretical calculations, high-resolution broadband rotational spectroscopy was recently proposed as a technique to investigate experimentally the conformations of the isolated, unperturbed molecule of ibuprofen in the gas-phase (Betz et al., 2015). Being the liquid phase the physiological environment where ibuprofen acts, it would be nevertheless interesting to study the molecular conformational equilibrium directly in solution. NMR spectroscopy has been proven to be a sensitive tool in this respect. Indeed, in the fast exchange limit, the measured NMR spectral parameters are averaged over the

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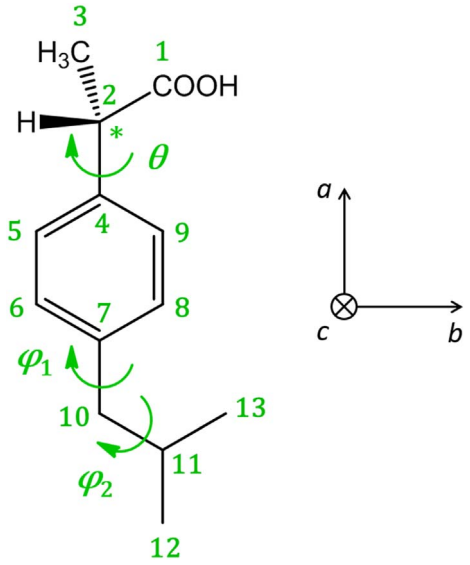

Fig. 1. Topological structure, carbon atoms labelling and torsional angles $\theta = H_2C_2C_4C_5$, $\varphi_1 = C_{11}C_{10}C_7C_6$ and $\varphi_2 = H_{11}C_{11}C_{10}C_7$ of *S*-ibuprofen (SIBU). Protons nuclei are numbered after the carbon they are bound to. The (a, b, c) axes of the molecular reference frame adopted for the molecules are also shown. The *c* axis, perpendicular to the (ab) plane of the aromatic ring, points behind the plane, defining a right-handed Cartesian system.

torsional internal motions, allowing the investigation of molecular flexibility. More importantly, the NMR studies of bioactive molecules can be carried out directly in a liquid phase, which is closer to physiological conditions with respect to the gas or the solid states. Standard NMR parameters used over the years for structural and conformational investigations are *J*-couplings and nuclear Overhauser effect (nOe) measurements (Luy et al., 2008). 2D nOe spectroscopy was recently applied to determine the preferred conformations of ibuprofen in deuterochloroform ($CDCl_3$) saturated solution (Khodov et al., 2014a). The aim was to get information on the conformational distribution in a saturated solution and thus shed light on the mechanism of formation of a certain crystal morphology within the nucleation process. Prominent differences in terms of relative population of the minimum energy conformers emerged from the comparison between the results obtained in saturated solution of $CDCl_3$ (Khodov et al., 2014a) and those obtained from quantum chemical calculations and vibrational spectroscopy (Vueba et al., 2008). As such studies pointed out, a change in conformer distribution moving from gas phase/unsaturated solution to saturated solution is not astonishing and it was already observed in similar cases (Khodov et al., 2014b). In the present work, we aim at experimentally probing the conformational space of ibuprofen in dilute organic solution by combining NMR spectroscopy with the use of anisotropic media. Unfortunately, standard NMR methods based on nOe measurements or *J*-couplings are limited to short-range connectivities and often do not provide enough unambiguous information for the structural and conformational elucidation of remote molecular fragments, particularly in the presence of multiple conformations in fast mutual exchange (Di Pietro et al., 2014). An appealing complementary strategy to access conformational information is applicable when the orientations of the solute molecules in the solution are not isotropically distributed, but rather statistically ordered (to a higher or lower extent) along a preferred direction, usually called “director”. This is what typically happens for NMR experiments in anisotropic solutions: the spectral parameters, called Residual Dipolar Couplings (RDCs), otherwise undetectable in isotropic phases due to their vanishing isotropic average, can then be observed and obtained from the NMR spectra. Physically speaking, the dipolar coupling constants D_{ij} originate from the through-space interaction between two magnetically active nuclear spins *i* and *j* and, according to the laws of magnetic interactions, depend on both the distance $\boldsymbol{r_{ij}}$ between them and the angle ϑ of the internuclear vector $\boldsymbol{r_{ij}}$ with respect to the external NMR magnetic field B_0. For flexible solutes (*i.e.* molecules endowed with internal torsional degrees of freedom), both $\boldsymbol{r_{ij}}$ and ϑ depend on the molecular conformation, so that the following expression holds for the $i-j$ RDC:

$$D_{ij} \propto \overline{[S_{ij}(\{\phi\})\cdot\langle r_{ij}^{-3}(\{\phi\})\rangle]}_{\{\phi\}} \quad (1)$$

where $S_{ij}(\{\phi\}) \equiv \left\langle \frac{3\cos^2\vartheta(\{\phi\})-1}{2} \right\rangle$ is the orientational order parameter and the symbol $\langle\ldots\rangle$ represents the statistical average over the so-called “molecular tumbling” (the overall molecular reorientational motion). The upper bar indicates an average, weighted over the complete conformational distribution of the flexible solute, $\{\phi\}$ being the set of torsional angles defining the entire conformational space sampled by the molecule (for more details, see ESI, Eq. (1), and refs. therein). Due to their geometrical dependence, the knowledge of experimental RDCs enables, in principle, the determination of the relative spatial arrangements of distant atoms of a molecule (Burnell and de Lange, 2003; Emsley, 1985, 2007; Gil, 2011; Kummerlöwe and Luy, 2009; Thiele, 2008) and, consequently, it allows to acquire significant information about structural molecular properties such as constitution, configuration and conformation(s) (Aroulanda et al., 2006; Celebre et al., 1992, 2006; De Luca et al., 2005; Emsley et al., 1994). The value of dipolar couplings to solve complex structure assignment problems is today recognized in pharmaceutical research (Liu et al., 2017). Their application also for the conformational analysis of small drugs sounds hence very appealing. The methodology was successfully applied to probe the conformational distributions of NSAIDs belonging to the family of salicylates and profens (Di Pietro et al., 2014, 2015). In an effort to gain a deeper insight on the conformational features of profens, we extend here the investigation to ibuprofen. The compound shows a stereogenic center at the α-carbon site connecting the carboxyl group and the aromatic ring (C_2 in Fig. 1) and it can then exist as an *R*(−) or an *S*(+) enantiomer. Although only the *S*(+) form is pharmaceutically active (Evans, 2001; Geisslinger et al., 1989), ibuprofen is commercially available as a racemic mixture, since *in vivo* the COX-inactive *R*(−) ibuprofen undergoes a unidirectional chiral inversion into the active *S*(+) form (Baillie et al., 1989; Tracy and Hall, 1992). Here we studied the biologically active *S*-ibuprofen (SIBU, Fig. 1).

As in previous studies (Di Pietro et al., 2014, 2015), the solvent used was the well-known weakly ordering chiral liquid crystal obtained by dissolving the synthetic homopolypeptide poly-γ-benzyl-L-glutamate (PBLG) in the organic $CDCl_3$ co-solvent (Samulski and Tobolski, 1968). Similar polymer - organic solvent mixtures were already applied in several NMR studies of small molecules (Aroulanda et al., 2001, 2003; Courtieu et al., 2002; Lesot et al., 2003, 2015; Rivard et al., 2003; Thiele and Berger, 2003) and also for a study of ibuprofen enantiomers using RDCs combined with theoretical modelling (Berger et al., 2012; Marathias et al., 2007). One of their main features is that the degree of orientational order experienced by a solute in such media is usually weak enough to give mainly high-resolution first order NMR spectra. Moreover, SIBU is poorly soluble in water, but it dissolves readily in such anisotropic phase. In the present study, the conformational distribution of SIBU in solution will be investigated starting from the experimental RDCs measured in a PBLG/$CDCl_3$ phase *via* the application of the robust theoretical approach known as the AP-DPD model (Celebre et al., 2003; Emsley et al., 1982). Results will be discussed also in the light of literature data obtained by molecular modelling calculations and other experimental methods.

2. Experimental and theoretical tools

2.1. Measurement of Residual Dipolar Couplings

The conformational study addressed here is based on the