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.

Keywords: conformation, NMR, 2D NOESY, ibuprofen (PubChem CID: 3672)

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 [37, 43, 52]. Experimental determination of spatial structure and conformational state of biologically active molecules attracts an increasing interest [9, 17, 19, 31].

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 [3]. It was firstly

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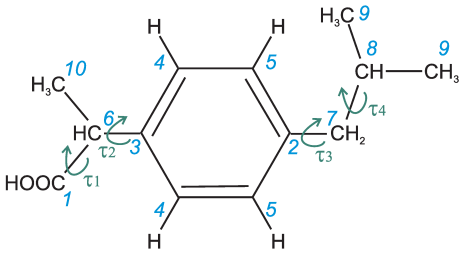

Figure 1: Scheme of the ibuprofen molecule with atom numbering and dihedral angles responsible for the formation of different conformers.

synthesized by Adams with his colleagues in 1961 and called BTS 13621. It has an outstanding biological activity among substituted phenylalkane and alkene acids [2, 3].

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 and co-authors have shown that only the S(+) form is pharmaceutically active [23]. The inactive R(−) ibuprofen, however, may undergo a unidirectional chiral inversion into the active S(+) form in vivo [23, 36].

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 [57] 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 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 [57] 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 crys-

Table 1: Dihedral angles determining differences between the ibuprofen conformers based on the quantum-chemical calculated structures [57]. Where equivalent atoms have the same name (C4, C5, and C9), one of them was chosen for analysis in all structures

Conformer	Dihedral angle (°)			
	$\tau 1$	$\tau 2$	$\tau 3$	$\tau 4$
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

talline phase II (melting point 290 K) was revealed in addition to the already known crystalline phase I (melting point 349 K) [15]. Its melting point is lower than that of the phase I and the Rietveld factors are high [12]. These observations, as well as Raman spectroscopy data [26], 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 [53].

It was shown in [44] 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 [9]; here we expanded this approach to the case of a multi-conformer molecule. The choice of solvent 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.

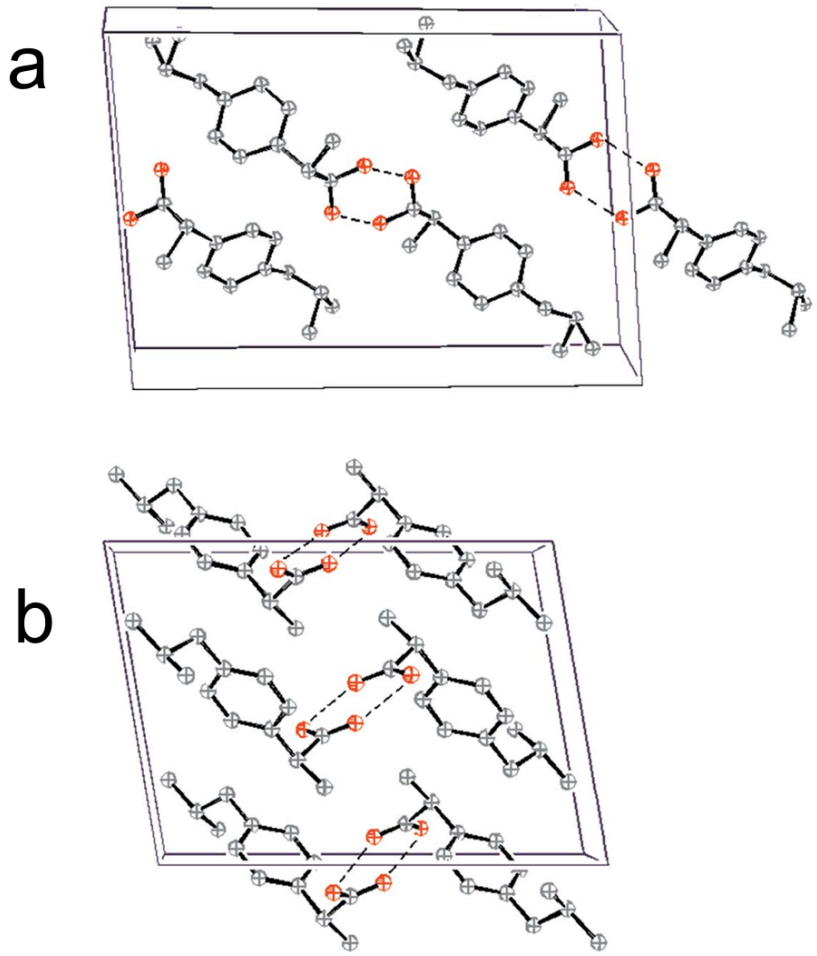

Figure 2: Unit cells of two ibuprofen conformers: (a) phase I [53] and (b) phase II [12].

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.

^{1}H 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) [4] 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 200 ms. The spectra were acquired with 8 scans and relaxation delay of 2 s.

Another pulse sequence used was the standard two-dimensional Incredible Natural Abundance DoublE QUAntum Transfer (2D ^{13}C-^{13}C INADEQUATE) [5] experiment including gradients to enhance pathway selection with 1024 points in the F2 direction and 128 points in the F1 direction. The spectrum was recorded with 96 scans and spectral widths of 200 ppm.

The two-dimensional ge-HMBC [29, 48] spectrum was recorded in a phase-sensitive mode using Echo/Antiecho-TPPI gradient selection with gradients in back-INEPT block. The spectrum was recorded with 2048 points in the F2 direction and 256 points in the F1 direction; 96 scans were acquired.

The two-dimensional ge-HSQC [42, 58] spectrum was recorded using gradient pulses for selection with 4096×256 data points (F2×F1); 16 scans per increment were acquired.

Acquisition parameters for HMBC, HSQC and INADEQUATE were calculated from coupling constants: ^1J(C,H) = 145 Hz, ^nJ(C,H) = 8 Hz, and ^1J(C,C) = 33 Hz.

Two-dimensional nuclear Overhauser effect spectroscopy experiments (2D ^{1}H-^{1}H ge-NOESY) [28] were performed with pulsed field gradient techniques [59] (Echo/Antiecho-TPPI mode, 2048×256). Mixing time values were 0.3, 0.45, 0.5, 0.6, 0.8, and 0.90 s. The spectra were acquired with 24 scans per increment; spectral width was 14 ppm; relaxation delay was 2 s.

2.2. Quantum Chemistry Calculations

The quantum mechanical calculations were carried out using the GAUSSIAN 03W (G03W) program package [20] within the Density Functional Theory (DFT) approach in order to properly account for the electron correlation

effects (particularly important in this kind of conjugated systems). The widely employed hybrid method denoted by B3LYP, which includes a mixture of HF and DFT exchange terms and the gradient-corrected correlation functional of Lee, Yang and Parr [34, 45], as proposed and parameterised by Becke [6, 7] was used, along with appropriate all-electron split valence basis sets. Molecular geometries were fully optimised (bond lengths to within ca. 0.1 pm and the bond angles to within ca. 0.1°) by the Berny algorithm, using redundant internal coordinates [49] and the 6–31G(d) basis set [25]. Computation of the harmonic vibrational wavenumbers confirmed eight different geometries as real minima on the potential energy surface of the molecule (no imaginary wavenumbers). The NMR shifts were determined within the GIAO methodology [14, 62] using the 6–311+G(2d,p) basis set [11, 33] and the previously optimized geometries [57]. Cheeseman et al. [10] found this model appropriate for reliable NMR predictions. In order to facilitate the comparison of the theoretical NMR shifts with the experimental values, the NMR spectrum of TMS was also calculated. Thus, the relative NMR shifts of each ibuprofen carbon (C_i) in relation to TMS, $\delta_{calc}(C_i)$, were determined in a similar way as performed experimentally,

$$\delta_{calc}(C_i) = \sigma(\mathrm{TMS}) - \sigma(C_i)\,,$$

where σ(TMS) and $\sigma(C_i)$ stand for the calculated isotropic magnetic shielding tensor of carbon of the reference (TMS) and of ibuprofen, respectively.

3. Results and Discussion

3.1. Experimental NMR Spectra of Ibuprofen

Assignment of all ^{1}H and ^{13}C NMR signals is necessary for comparing the results of quantum chemical calculations with experimental ^{13}C NMR data and correct interpretation of NOESY data.

Assignment was verified by combination of ^{13}C NMR data and 2D correlation spectra HMBC, HSQC, TOCSY, and NOESY. The HMBC and HSQC spectra, however, can be interpreted in two ways. One ^{13}C assignment is that also reported in [27, 40, 63], while the other (which is supported by NOESY and TOCSY spectra) coincides with results in [1, 8, 41]. To clarify which literature data is correct, we performed a (^{13}C,^{13}C)-INADEQUATE experiment (Fig. 3).

Analysis of the INADEQUATE spectrum allowed us to trace the connectivities of carbon atoms: C9-C8-C7-C2-C5-C4-C3-C6. Thus, complete assignment of signals in ^{1}H and ^{13}C NMR spectra of ibuprofen in chloroform was achieved. ^{13}C chemical shifts are presented in the Supplementary Info, Table S1.

3.2. Comparison of Experimental and Simulated ^{13}C NMR Data

Linear correlations between the observed ^{13}C chemical shifts (δ) and the GIAO shielding constants (σ) were built according to the equation $\delta_{exp}(C_i) = a + b\delta_{calc}(C_i)$ (such an approach was employed in [47, 55]). Calculated chemical shifts for different conformers are listed in Table S1; correlations are plotted in Fig. 4.

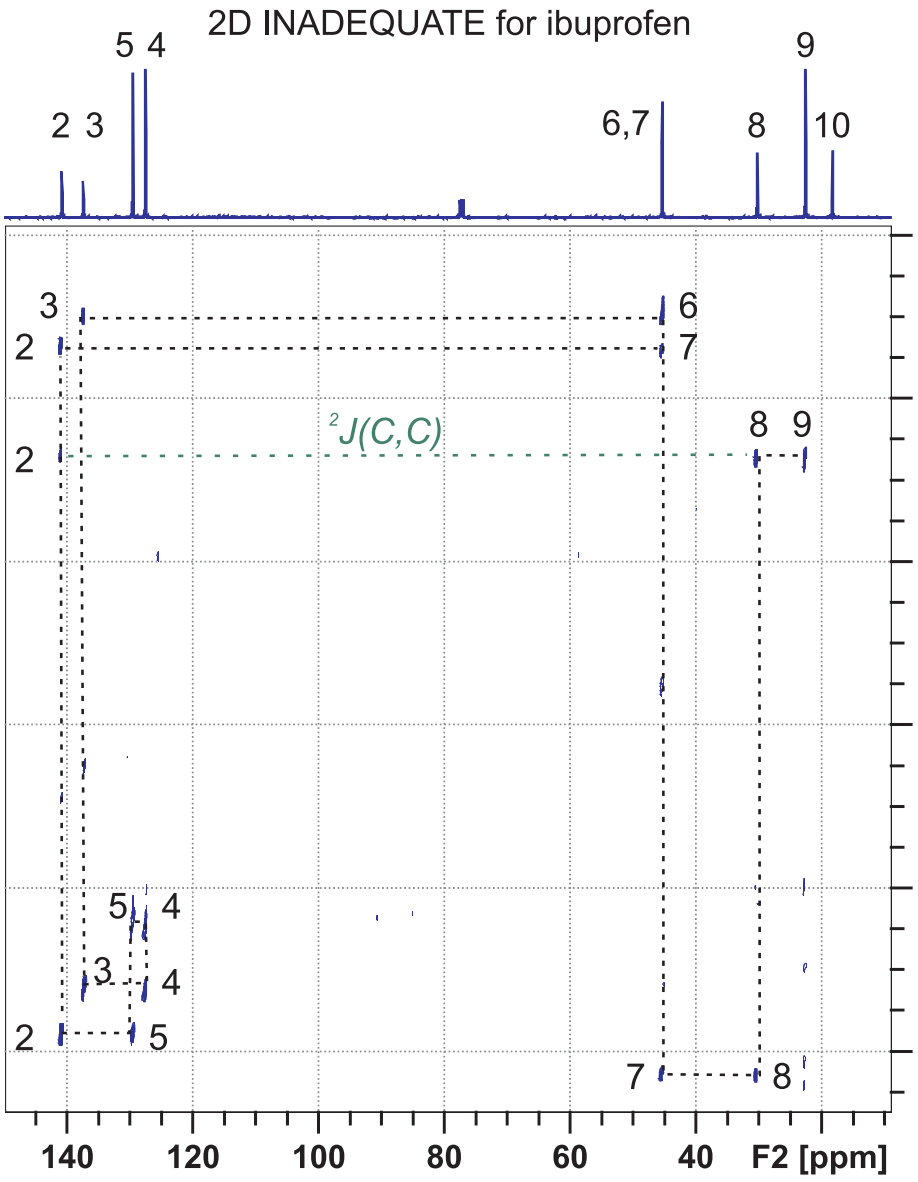

Figure 3: (^{13}C,^{13}C)-INADEQUATE spectrum of ibuprofen dissolved in $CDCl_3$ (observe frequency is 125 MHz). Dotted lines show the correlations between consecutive carbon atoms in the molecule.

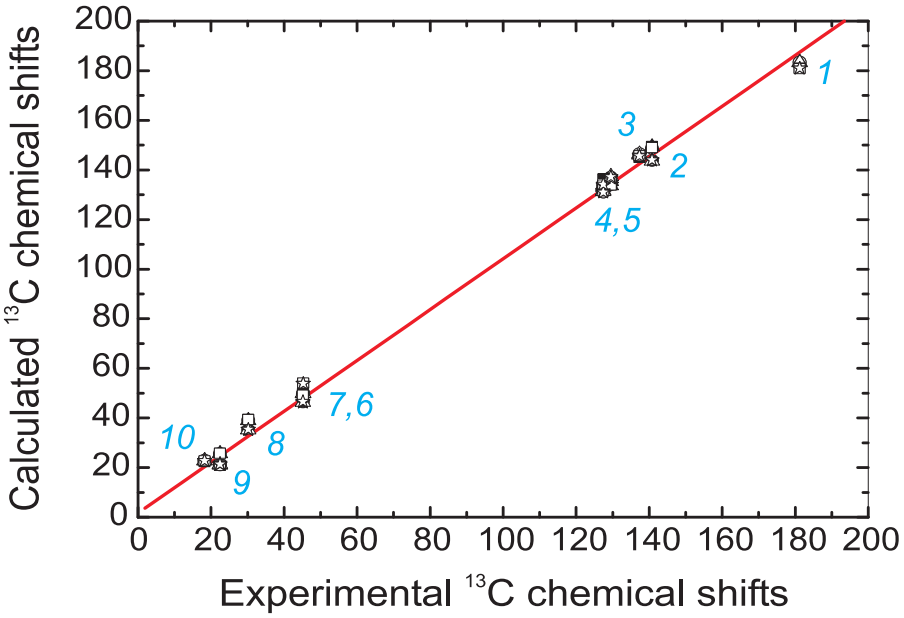

Figure 4: Correlations between theoretical ^{13}C NMR chemical shifts of all conformers of ibuprofen (A–H) and observed chemical shifts in chloroform. Numbers designate the carbon atoms (see Fig. 1). Difference between the conformers is evidently small, and all calculated values agree quite well with the measured chemical shifts.

When a fast conformational exchange takes place in the system, observed chemical shifts are weighted average of the δ values of individual conformers, according to the relation

$$\delta_{exp}(\mathrm{C}_i) = a + b \sum_k p_k \delta_k^{calc} ,$$

where k enumerates all conformers, and p_k are their relative fractions. In principle, comparison of theoretical and measured chemical shifts gives a possibility to calculate fractions p_k of conformers in exchange. However, in practice this usually leads to an ill-posed problem, since differences between chemical shifts of the same atoms in different conformers are small.

Difference between calculated chemical shifts for different conformers of ibuprofen achieves 4 ppm for some atoms, but it is not enough to find reliable quantity of all conformers A–F. Correlations $\delta_{exp} - \delta_{calc}$ for all conformers show R^2 from 0.996 to 0.998, and hence cannot be used for choosing any of them as the preferred conformer in solution. For this reason, we used an alternative approach based on the nuclear Overhauser effect spectroscopy.

3.3. Determination of Conformer Fractions by 2D NOESY

Two-dimensional nuclear Overhauser effect spectroscopy is a powerful method of studying spatial structure and conformation of molecules in solution. Intensities of diagonal and cross-peaks in a 2D NOESY spectrum can be gathered in a matrix $\mathbf{A}$, which depends on the mixing time τ_m (see Fig. 5) as follows:

$$\mathbf{A}(\tau_m) = \exp(-\mathbf{R}\tau_m)\mathbf{A}_0 .$$

Here $\mathbf{A}_0$ corresponds to the zero mixing time; its elements a_{0i} are proportional to the equilibrium populations of individual spin states: $a_{0i} = n_i a_0$. The number of equivalent nuclei is designated n_i (e.g., $n_i = 3$ for methyl groups); a_0 is the intensity corresponding to a single spin state.

$\mathbf{R}$ is the cross-relaxation matrix [39, 38]:

$$\mathbf{A} = \begin{pmatrix} \rho_{11} & \sigma_{12} & \cdots & \sigma_{1n} \\ \sigma_{21} & \rho_{22} & \cdots & \sigma_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ \sigma_{n1} & \sigma_{n2} & \cdots & \rho_{nn} \end{pmatrix} ,$$

where matrix elements ρ_{ij} and σ_{ij} are the longitudinal and cross-relaxation rates. For the general case of an N-spin system they are given by the following expressions:

$$\sigma_{ij} = \frac{1}{10}\hbar^2\gamma^4 \left(\frac{\mu_0}{4\pi}\right)^2 \sum_{i=1, i\neq j}^{N} \frac{n_i}{r_{ij}^6} \left(6J^2(\omega) - J^0(\omega)\right) ,$$

$$\rho_{jj} = \frac{1}{10}\hbar^2\gamma^4 \left(\frac{\mu_0}{4\pi}\right)^2 \sum_{i=1, i\neq j}^{N} \frac{n_i}{r_{ij}^6} \left(6J^2(\omega) + 3J^1(\omega) + J^0(\omega)\right) .$$

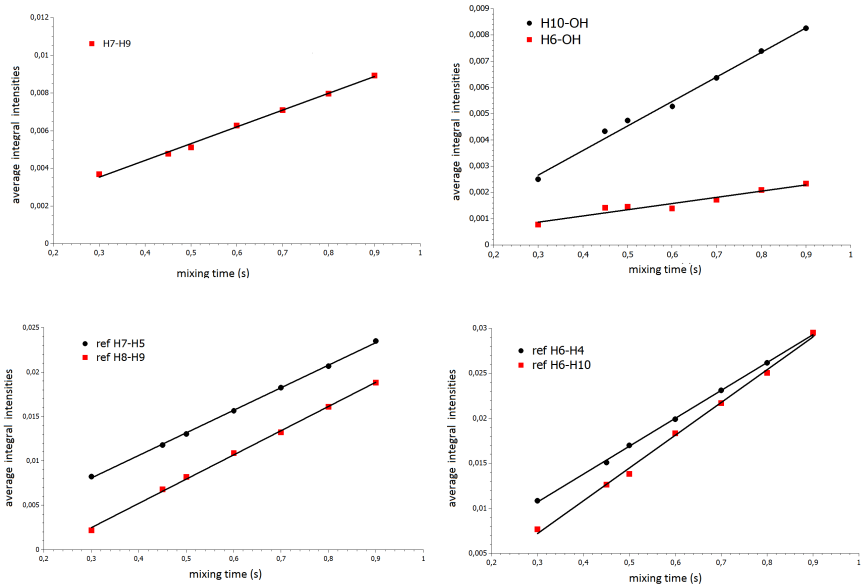

Figure 5: Build-up dependencies of averaged normalized integral intensities of cross-peaks in 2D NOESY spectra of ibuprofen.

$J(\omega)$ is the spectral density function, which for the case of isotropic tumbling of the molecule has the form

$$J^m(\omega) = \frac{\tau_c}{1 + m^2\omega^2\tau_c^2}\,.$$

Here τ_c is the correlation time, which is on the order of 10^{-10} s for small molecules; m stands for the order of relaxation transitions (0, 1, or 2).

As was shown in [18, 21, 61], where series expansion into powers of $\exp(-\mathbf{R}\tau_m)$ is used, dependency of the averaged integral intensity $\langle I\rangle$ on the mixing time τ_m

$$\langle I(\tau_m)\rangle = \frac{1}{2}\left(\frac{1}{n_j}\left|\frac{a_{ij}(\tau_m)}{a_{ii}(\tau_m)}\right| + \frac{1}{n_i}\left|\frac{a_{ji}(\tau_m)}{a_{jj}(\tau_m)}\right|\right)$$

may with a high degree of accuracy be considered linear with the τ_m values much higher than those usually employed in the initial rate approximation.

Determination of the distance between different protons is based on a strong dependence of cross-relaxation rates σ_i on distances r_i between them. Usually this dependency is approximated by a simple formula

$$\sigma_i \sim \frac{n_i\tau_c}{r_i^6}\,;$$

in this case, distances are obtained from the expression (1) using a known reference distance r_{ref}:

$$r_i = r_{ref}\left(\frac{\sigma_{ref}}{\sigma_i}\right)^{1/6}. \tag{1}$$

Interproton distances were found based on experimentally obtained cross-relaxation rates. Different proton pairs, most suitable for a studied molecular fragment, were chosen as the calibration pairs.

Intramolecular motion and magnetic equivalence of atoms should be taken into account when averaging theoretical distances in order to compare theoretical and experimentally found internuclear distances. In the case of slow motion, effective distances may be calculated following the formula

$$r_i^{eff} = \left[\frac{1}{n_I n_S}\sum_i \frac{1}{r_i^6}\right]^{-1/6}. \tag{2}$$

It is used in most cases of calculating proton-proton distances. Koning and co-authors showed [32] that flips of a benzene ring may be allowed for in Eq. (2) by averaging two equivalent protons on the both sides of the benzene ring.

In the case of fast motion, when angular oscillations can be neglected, effective distances are obtained as follows:

$$r_i^{eff} = \left[\frac{1}{n_I n_S}\left(\sum_i \frac{1}{r_i^3}\right)^2\right]^{-1/6}. \tag{3}$$

Motion of protons in the methine-methylene (CH–CH_2) and phenyl-methylene (C_6H_5–CH_2) systems falls within the field of application of Eq. (3). Sometimes this expression is used for averaging distances in the methine-methyl (CH–CH_3) group. However, this way for determining interproton distances is an approximate estimation.

Intramolecular motion of methyl groups (with characteristic time of ∼50 ps) is much faster than the overall tumbling of a molecule (200–300 ps). Positions of atoms of methyl groups should be averaged because of their fast rotation and magnetic equivalence. Tropp proposed a model describing the motion of a methyl group, which considers each of the protons independently [56]. According to this model, change of the position has a jump-like nature. Number N of these position changes can take a value of 0, 3, 6, 12, etc. Choice of a certain value for N depends on the ease of rotation of CH_3 groups. For the case of unrestrained motion, $N > 3$; if protons jump between three positions, $N = 3$; and if the methyl group is static, $N = 0$. Cases of theoretical averaging assuming $N = 6$ or 12 show the relative difference in the NOE values less than 0.001 [16]. Use of $N > 12$ is therefore inexpedient. Difference between the cases $N = 3$ and 6 is also small, about 0.01, which falls within the error of the NOESY method. For the sake of simplicity, we used the model with $N = 3$:

$$r_{eff} = \left[\frac{1}{5} \sum_{k=-2}^{2} \left| \frac{1}{3} \sum_{i=1}^{3} \frac{Y_{2k}(\theta^i_{mol}, \varphi^i_{mol})}{r_i^3} \right|^2 \right]^{-1/6} . \qquad (4)$$

where $Y_{2k}(\theta^i_{mol}, \varphi^i_{mol})$ are the second rank spherical harmonics. Equation (4) is applied for each of the protons of the benzene ring and of the methyl group. Afterwards, effective distance between mentioned group of atoms is found using Eq. (2). In the case of the methylene-methyl system, effective interproton distance is obtained by averaging (4) for all protons in the CH_3 group and each individual proton in the CH_2 group; and then calculated distances are averaged following Eq. (3).

Average distances obtained using Eqs. (2), (3), and (4) are compared in Table 2. A systematical difference between the results given by these three models is evident: averaging according to Eq. (2) underestimates the distances, while the values obtained within the model of spherical harmonics are the closest to the experimentally obtained distances r_{exp}.

It is evident also from the table that correlation between calculated and experimentally found distances is quite well for conformers G and H and poor for conformers A–F.

Use of described above averaging procedure is necessary for exact determination of conformational state of small flexible bioactive molecules, based on comparison of experimental and theoretical values of internuclear distances.

Lee and Krishna showed that for the case of fast conformation exchange, the net cross-relaxation rate is the weighted average of rates corresponding to individual conformers [35]:

$$\sigma = \sum_i \sigma_i x_i \,.$$

Following this relation, one can obtain the dependence of conformer fractions on distances for the case of a two-position exchange:

$$\frac{1}{r_{exp}^6} = \frac{x_1}{r_1^6} + \frac{1-x_1}{r_2^6} \,,$$

$$x_1 = \frac{r_1(r_2^6 - r_{exp}^6)}{r_{exp}^6(r_2^6 - r_1^6)} \,, \quad x_2 = \frac{r_2(r_{exp}^6 - r_1^6)}{r_{exp}^6(r_2^6 - r_1^6)} \,.$$

All conformers were divided into two groups with respect to atom-atom distances; each of these was then divided into two sub-groups. The first group, A–D, is defined by common values of distances H6–H10, H6–H4, OH–H4, which are influenced by rotations $\tau 1$ and $\tau 2$ (see Fig. 1). The remaining forms E–F are united in the second group. The dihedral angles $\tau 3$ and $\tau 4$ define the subgroups (A, B, E, F) and (C, D, G, H).

Concerning the interatomic distances, one should account for spin diffusion. This phenomenon leads to underestimation of the distance due to the presence of additional magnetization transfer routes, and even leads to appearing of cross-peaks in NOESY spectra for atoms that are far away from each other and should not show a cross-peak (e.g., the cross-peak between the atoms OH1 and H5, see Fig. 6). Thus, we analyzed three pairs of protons that are free from intermediate protons, which might provide the spin diffusion and bias the distance values: OH1–H10, OH1–H6, and H7–H9. The former two give the criterion for distinguishing groups of conformers (A–D) and (E–F), and the latter one allows to distinguish the groups (A,B,E,F) and (C,D,G,H). Distances H6–H4, H6–H10, H7–H5, and H8–H9 served as calibration distances. Choosing either of these did not alter the calculated distances, which proves the correctness of the analysis.

It is evident from Table 2 that the experimental distances OH1–H10 and OH1–H6 are close to the values describing the groups of conformers (E–H), which thus should dominate in solution to a degree of ∼90%. In a similar way, r_{exp}(H7–H9) shows that fraction of conformers (C,D,G,H) is larger than that of (A,B,E,F). Combining the two criteria together, we conclude that (G,H) have the biggest fraction of all conformers coexisting in the saturated solution of ibuprofen in chloroform. The fractions of the conformer pairs (A,B), (C,D), (E,F), and (G,H) are approximately 1, 5, 9, and 85% (Fig. 7). More precise estimates of the fractions will require more accurate measurements of effective distances; some effort towards eliminating the influence of spin diffusion may be needed.

Comparing results of vibrational spectroscopy and quantum chemical calculations, authors of [57] note that it is possible to be limited to only four different conformers (see Introduction). Thus, we will present the results considering four pairs of conformers, so that conclusions could be easily compared. Boltzmann's distribution of the conformers in vacuum at room temperature is

Table 2: Distances between analyzed pairs of atoms in the ibuprofen molecule calculated within different averaging models (Eqs. (2–4)). Experimental distances are obtained from analysis of NOESY data (Fig. 5). In cases when different models turn out to be identical, which depends on the motion type, distances are not listed

protons	confor-	Spher.	ˆ3	ˆ6	protons	confor-	Spher.	ˆ3	ˆ6
	mers	harm.	Eq.	Eq.		mers	harm.	Eq.	Eq.
r_{exp}, Å		Eq. (4)	(3)	(2)	r_{exp}, Å		Eq. (4)	(3)	(2)
H6–H10	A	2.72	2.65	2.62	H8–H9	A	2.68	2.64	2.61
	B	2.71	2.65	2.62		B	2.68	2.64	2.61
2.72 ± 0.04	C	2.73	2.65	2.62	2.67 ± 0.06	C	2.67	2.63	2.60
reference	D	2.72	2.65	2.62	reference	D	2.67	2.63	2.60
CH–CH_3	E	2.70	2.64	2.61	$CH_2(CH_3)_2$	E	2.68	2.64	2.61
	F	2.71	2.64	2.61		F	2.68	2.64	2.61
	G	2.71	2.64	2.61		G	2.67	2.63	2.60
	H	2.72	2.64	2.61		H	2.67	2.63	2.60
H6–H4	A	–	2.74	2.59	H7–H5	A	–	2.88	2.76
	B	–	2.74	2.59		B	–	2.88	2.76
2.70 ± 0.04	C	–	2.73	2.59	2.75 ± 0.03	C	–	2.86	2.75
reference	D	–	2.74	2.59	reference	D	–	2.87	2.75
CH–CH_2	E	–	2.71	2.56	CH_2–CH_2	E	–	2.88	2.76
	F	–	2.70	2.55		F	–	2.88	2.76
	G	–	2.71	2.55		G	–	2.87	2.75
	H	–	2.71	2.56		H	–	2.86	2.75
H6–OH1	A	–	–	3.37	H7-H9	A	3.19	3.15	1.90
	B	–	–	3.37		B	3.19	3.14	1.90
	C	–	–	3.37		C	3.43	3.38	2.03
3.89 ± 0.08	D	–	–	3.37	3.42 ± 0.06	D	3.43	3.38	2.03
CH–CH	E	–	–	3.95	$CH(CH_3)_2$	E	3.19	3.15	1.90
	F	–	–	3.94		F	3.19	3.14	1.90
	G	–	–	3.94		G	3.43	3.38	2.03
	H	–	–	3.94		H	3.43	3.38	2.03
H10–OH1	A	4.82	4.69	4.66					
	B	4.80	4.69	4.66					
	C	4.82	4.69	4.65					
4.09 ± 0.09	D	4.81	4.69	4.65					
CH_3–CH	E	4.04	3.95	3.88					
	F	4.05	3.95	3.88					
	G	4.04	3.95	3.88					
	H	4.06	3.95	3.88					

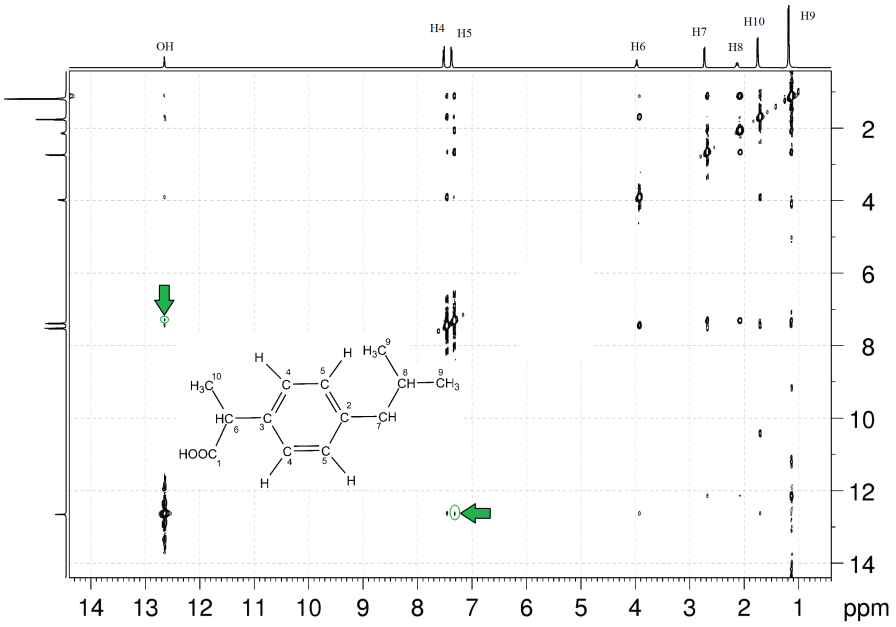

Figure 6: 2D NOESY spectrum of ibuprofen (500 MHz). Arrows point to the peaks arising due to spin diffusion.

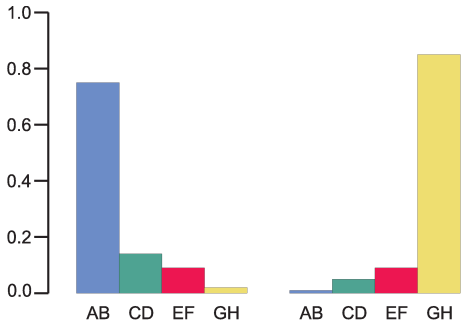

Figure 7: Distribution of ibuprofen conformers according to their calculated energies (Boltzmann's distribution [57], left panel) and obtained from analysis of NMR data (right panel).

Table 3: Comparison of NOESY and X-ray data [12] with respect to the conformation of the propionic acid fragment

Relevant dihedral angle	Phase I[a]	Phase II[b]	Conf. A,B,C,D	Conf. E,F,G,H
$\tau 1$	114.6	−88.7	89.2	−81.1

[a] Based on X-ray data. [b] Based on NOESY data.

as follows: 75.0% (A+B), 14.0% (C+D), 9.0% (E+F), and 2.0% (G+H) (Fig. 7, left diagram).

Difference of the distribution of ibuprofen conformers in solution from that obtained by quantum chemical calculations (which were proved by vibrational spectroscopy [57]) is rather prominent. The same change in the character of conformer distribution takes place for felodipine in DMSO upon transition from an unsaturated solution to the saturated one [30]. Similar changing in distribution arising due to intermolecular interactions was observed in chiroptical spectroscopy experiments for chiral binaphthyl derivatives [13] and in MD simulations of n-butane in a confined medium [24].

Table 3 illustrates results of comparison of NOESY and X-ray data [12]. Conformations A, B, C, and D, which dominate in unsaturated solutions (and in gas phase), resemble the molecular structure of the polymorph phase I, while forms E, F, G, and H, which dominate in the saturated chloroform solution, are close to the phase II structure.

4. Conclusions

We showed on the example of the ibuprofen–chloroform system that NOE spectroscopy allows to estimate the conformational distribution of small molecules in solution, where approach based on comparison of theoretical and observed ^{13}C chemical shifts may fail. It was revealed that conformers G and H dominate in the saturated solution of ibuprofen in chloroform. The difference in distributions obtained from the quantum chemistry and analysis of NOESY data can be attributed to the solvent effect and intermolecular interactions.

The groups of conformers (A–D) and (E–H) were found to resemble the crystalline phases I and II of ibuprofen. The fact that the conformers (G,H) dominate in the saturated solution in chloroform may be of practical use when questions of nucleation of certain polymorphs are concerned. Phase I of ibuprofen can readily be obtained at ambient temperature by evaporation of the chloroform solution [60]. However, the dominant conformer in the saturated solution is found to be as the main structural unit of the phase II. This apparent discrepancy may be explained by the metastable nature of the phase II which transforms very quickly to the phase I. A similar behavior was observed recently for another low-molecular-weight molecule, D-mannitol [54]: the stable β form of mannitol can be obtained from low-concentration solutions, whereas the metastable α form is produced in the nucleation process at high concentrations. Moreover, recent investigation of crystal formation [46] argue in favour of

the existence of a more complex two-step mechanism by which molecules first condense into a metastable form which then evolves into a denser crystalline structure. Such a two-step process in the case of small organic molecules was also proved by analysis of experimental data in [22] and molecular dynamics simulation [51, 50]. The same may be suggested for nucleation of ibuprofen from chloroform.

Though the distribution of conformers was estimated, there are some atom-atom distances which might be useful for more accurate determination of conformer fractions but cannot be found straightforwardly due to the multistep magnetization transfer (spin diffusion). It is worse doing to find the way of overcoming this obstacle. In addition, discrepancies in signal assignment sometimes even for relatively small molecules such as ibuprofen may become a source of erroneous conclusions, and this aspect of NMR spectroscopy also needs a careful treatment.

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