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The role of hydrogen bonds in an aqueous solution of acetylsalicylic acid: a molecular dynamics simulation study

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Abstract This work focuses on the role of the dynamic hydrogen bonds (HB) formed in an aqueous solution of aspirin using molecular dynamics simulation. The statistics reveal the existence of internal HB that inhibit the rotational movements of the acetyl and the carboxylic acid groups, forcing the molecule to adopt a closed conformer structure in water, and playing an important role in stabilizing this conformation.

Keywords Hydrogen bond · Aspirin · Aqueous solution · Molecular dynamics simulation

Introduction

Hydrogen bonds (HB) are important in determining the conformation of many types of biopolymers. The present work is focused on the role of the dynamic hydrogen bonds that exist in an aqueous solution of acetylsalicylic acid. We assessed the role of HB by molecular dynamics (MD) simulation, which has proved a highly reliable technique with which to predict the properties of molecules in solution [1–4]. The aim of this study dealing with aspirin in aqueous solution is two-fold: (1) to gain a better understanding of the role played by dynamic intramolecular hydrogen bonds in determining the conformation of molecules in aqueous solution, and (2) to gain a better understanding of the molecular structure of acetylsalicylic acid—aspirin—a widely known anti-inflammatory drug [5]. Some authors [6] have suggested that the presence of intramolecular HB in the ortho-hydroxybenzoic acid and its derivatives (aspirin is the most representative molecule of this group) is essential if these compounds are to have antirheumatic properties. The current contribution demonstrates the existence of intramolecular HB between neighboring aspirin groups. Intramolecular HB have also been found by ab initio studies [7], and other work by Ouvrard and Price [8] based on the analysis of aspirin gas phase conformers and multiple searches for minima in the lattice energy [8]. These internal HB could also play an important role in stabilizing the molecular conformation of aspirin.

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Methods and models

The MD simulations were performed using the GROMOS 96 [9] package, which comes with the GROMOS 96 Force field ([9], p I-13). The GROMOS 96 Force field has proved reliable for the simulation of small molecules in solution [1–4], despite the fact that it was developed for applications involving aqueous or apolar solutions of proteins, nucleotides and sugars ([9], p I-13).

All MD runs were made at constant pressure and constant temperature, maintaining 1.013×10^5 Pa and 300 K, respectively. The temperature was kept constant by coupling the system to a Berendsen's thermal bath [10] while the pressure was controlled by a Berendsen's barostat. The integration time step was 0.001 ps, and simulation continued for 500 ps after equilibrium was reached. To test the influence of the water environment, MD simulations in vacuum were also performed. Vacuum simulations were done on a free volume while the solution

was simulated in a rectangular box (1.90 nm×2.17 nm×2.30 nm) with periodical boundary conditions. In the simulation box, 1 aspirin molecule was surrounded by 318 molecules of water. In the current model of aspirin (Fig. 1), the benzene ring is kept rigid in a planar conformation, applying improper torsion potentials that avoid transitions among other possible conformations. The particular geometry of carbons is also maintained by improper torsion potential. Bond lengths were maintained rigid, and bond angles were treated as harmonic potentials with their respective values taken from the GROMOS96 43a1 force field [9]. Charges were generated with the Spartan 02 [11] program, using Mulliken population analysis, while water was modeled using the SPC/E model [12]. Analysis of the properties of the system, movement of the molecules (dihedral angles) and interactions (HB) were done using a combination of GROMOS utilities and our own programs.

Results and discussion

Flexibility

Rotation around the side groups was taken as a measure of the mobility of aspirin. The relative mobility of the side groups has no restrictions other than those imposed by mutual atom interactions. Following this criterion, the trajectories described by the dihedral angles Φi, with i=1–5 (Φ1,Φ2, Φ3, Φ4 and Φ5) involved within the ring and the side groups were recorded. Figures 2 and 3 show such trajectories for the two different cases: in vacuum (V) and in water solution (W). In general, rotational dynamics is a very sensitive probe of the interatomic forces in crystals.

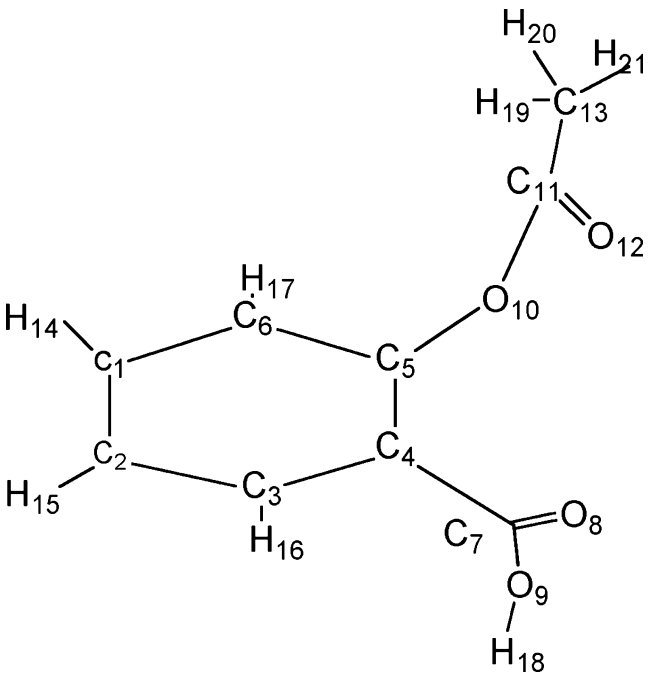

Fig. 1 Current model of acetylsalicylic acid (aspirin)

Analysis in vacuum

"Simulating a molecular system in vacuum, that is without any wall or binding corresponds to the gas phase at pressure zero ([9], Chap. 2.8.2, p II-99)". The shielding effect of a solvent with high dielectric permittivity, such as water, or the electric interaction between charges or dipoles in a molecule, is lacking under vacuum. The dihedral angles for the aspirin neighboring groups in vacuum: $\Phi 1_v$, $\Phi 2_v$ and $\Phi 3_v$ (with $\Phi 1_v = C_6–C_5–O_{10}–C_{11}$,$\Phi 2_v = C_5–C_4–C_7–O_8$ and $\Phi 3_v = C_5–O_{10}–C_{11}–O_{12}$) are shown in Figs. 2a, 2b and 2c, respectively. These dihedral angles fix the orientation of the side groups. Figures 2d and 2e refer to the $\Phi 4_v$ and $\Phi 5_v$ dihedral angles ($\Phi 4_v = C_4–C_7–O_9–H_{18}$ and $\Phi 5_v = O_{10}–C_{11}–C_{13}–H_{19}$), which are related to the exocyclic and methyl groups, respectively. The movement of the acetyl group is governed mainly by the trajectories of $\Phi 1_v$ and $\Phi 3_v$. Their average values are $\langle\Phi 1\rangle_v = \pm 90°$, $\langle\Phi 3\rangle_v = \pm 120°$. For both angles, two conformations were accessed during the 500 ps interval spanned. The orientation of the carboxylic group, $\Phi 2_v$, fluctuates between $\langle\Phi 2\rangle_v = +180°$ and $\langle\Phi 2\rangle_v = 0°$. From $\Phi 4_v$, the hydroxyl group was seen to have a preferred fix orientation about $\langle\Phi 4\rangle_v = +180°$, with some jumps to $\Phi 4_v = -180°$, while from $\Phi 5_v$, the hydroxymethyl group seems to rotate freely ($\langle\Phi 5\rangle_v \pm 180°$).

Analysis in water

The side group supported by the $C_5–O_{10}$ bond (Fig. 3a) is almost quiet ($\langle\Phi 1\rangle_w = \langle C_6–C_5–O_{10}–C_{11}\rangle = 90°$), thus, there are no rotational movements around the $C_5–O_{10}$ bond. This group is fixed during the entire simulation at the conformation of $\langle\Phi 1\rangle_w = 90°$. The carboxylic acid and the acetyl groups (Fig. 3b,c) have restricted movements. Both of them have two conformations ($\langle\Phi 2\rangle_w = \langle C_5–C_4–C_7–O_{8>}\rangle = \pm 100°$ and ($\langle\Phi 3_w = \langle C_5–O_{10}–C_{11}–O_{12}\rangle = \pm 75°$), which fix the orientation of the neighboring groups. The hydroxyl group ($O_9–H_{18}$ in Fig. 1), was found to prefer a particular orientation at values of $\langle\Phi 4\rangle_w \pm 60°$ for the torsion angle, $\Phi 4_w = C_4–C_7–O_9–H_{18}$ (Fig. 3d). This conformation could allow the formation of an intramolecular HB between the hydroxylic hydrogen, H_{18}, and the carboxylic oxygen, O_{12}. It seems that internal HB (Table 1) are favored by particular orientations of the hydroxyl group. The external H_{19} atom of the methyl group (Fig. 3e) (also H_{20} and H_{21}) rotates freely ($\langle\Phi 5\rangle_w = \langle O_{10}–C_{11}–C_{13}–H_{19}\rangle = \pm 180°$). Thus, these hydrogen atoms have no opportunity to be involved in any HB pattern.

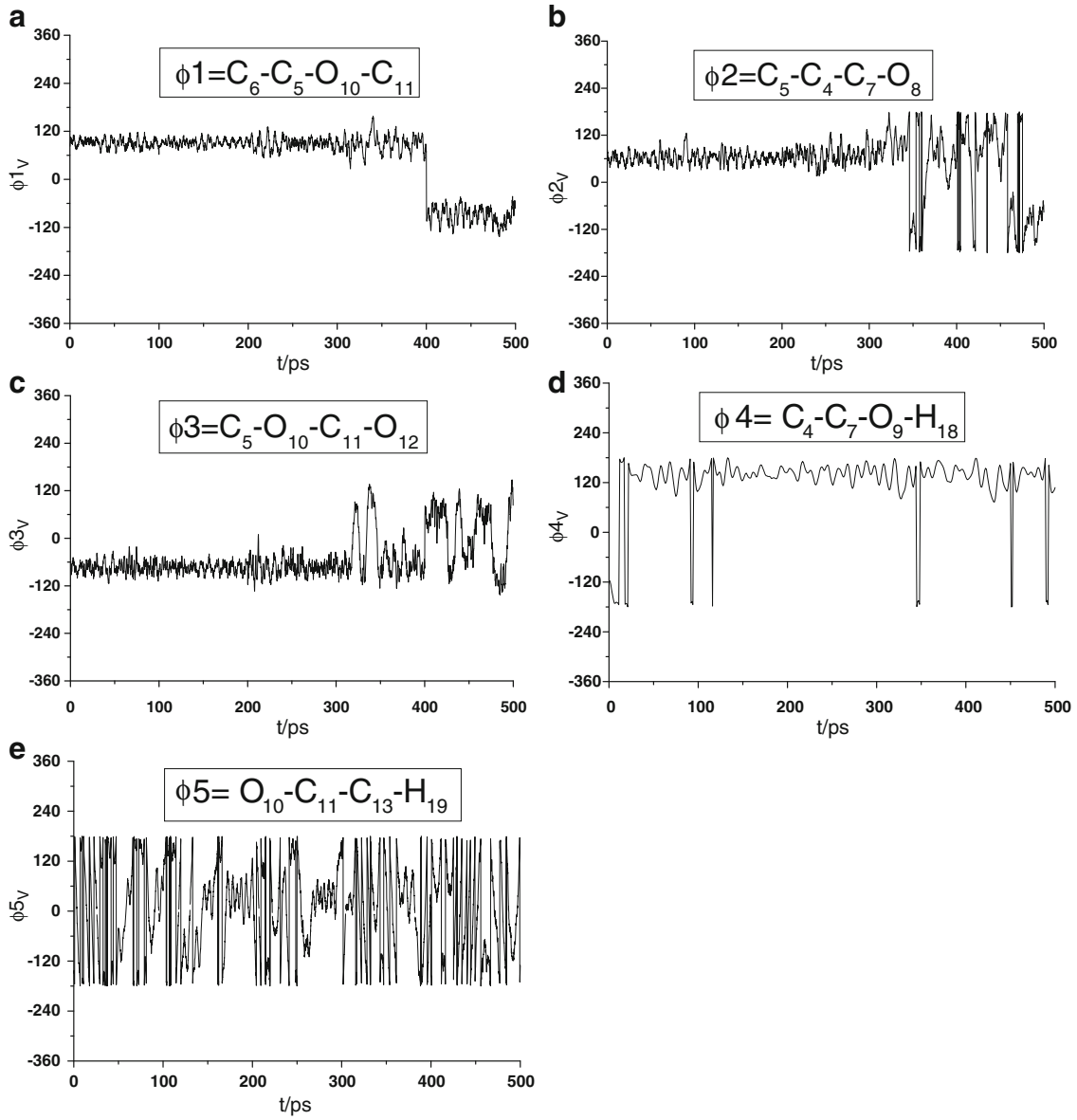

Fig. 2a–e Temporal trajectory evolution in vacuum (V). *x-axis* Time (ps), *y-axis* dihedral angles Φi, with i=1–5 (**a** Φ1, **b** Φ2, **c** Φ3, **d** Φ4 and **e** Φ5)

Internal hydrogen bonds

To analyze HB in the system, we used the programs PROAHB and PROCHB in the Gromos 96 package [9]. For the computation of HB we used a geometric criterion in which the bond is considered to be formed when (1) the distance between the acceptor and the proton is less than 0.24 nm (nanometer), and (2) the O–H–O angle lies between 145° and 180° [9, 13].

The results show that there are no HB in vacuum. This may be ascribed to the fact that water is a solvent with high electric permittivity, and also there are many interactions between water molecules and the test molecule, resulting in very high repulsive energies in the system. The standard procedure to remove these close contacts and interactions is to perform energy minimization (EM) of the system before running MD simulations. The EM procedure changes the coordinates of the molecule in order to minimize the energy of the system. This particular spatial arrangement of the aspirin in water favors HB formation, as it fulfils the geometric criterion mentioned above.

MD calculations in aqueous solution (Table 1) show the existence of intramolecular HB between the two lateral groups, and the acetyl and carboxylic acid groups calculated over the 500 ps spanned. These HB-mediated

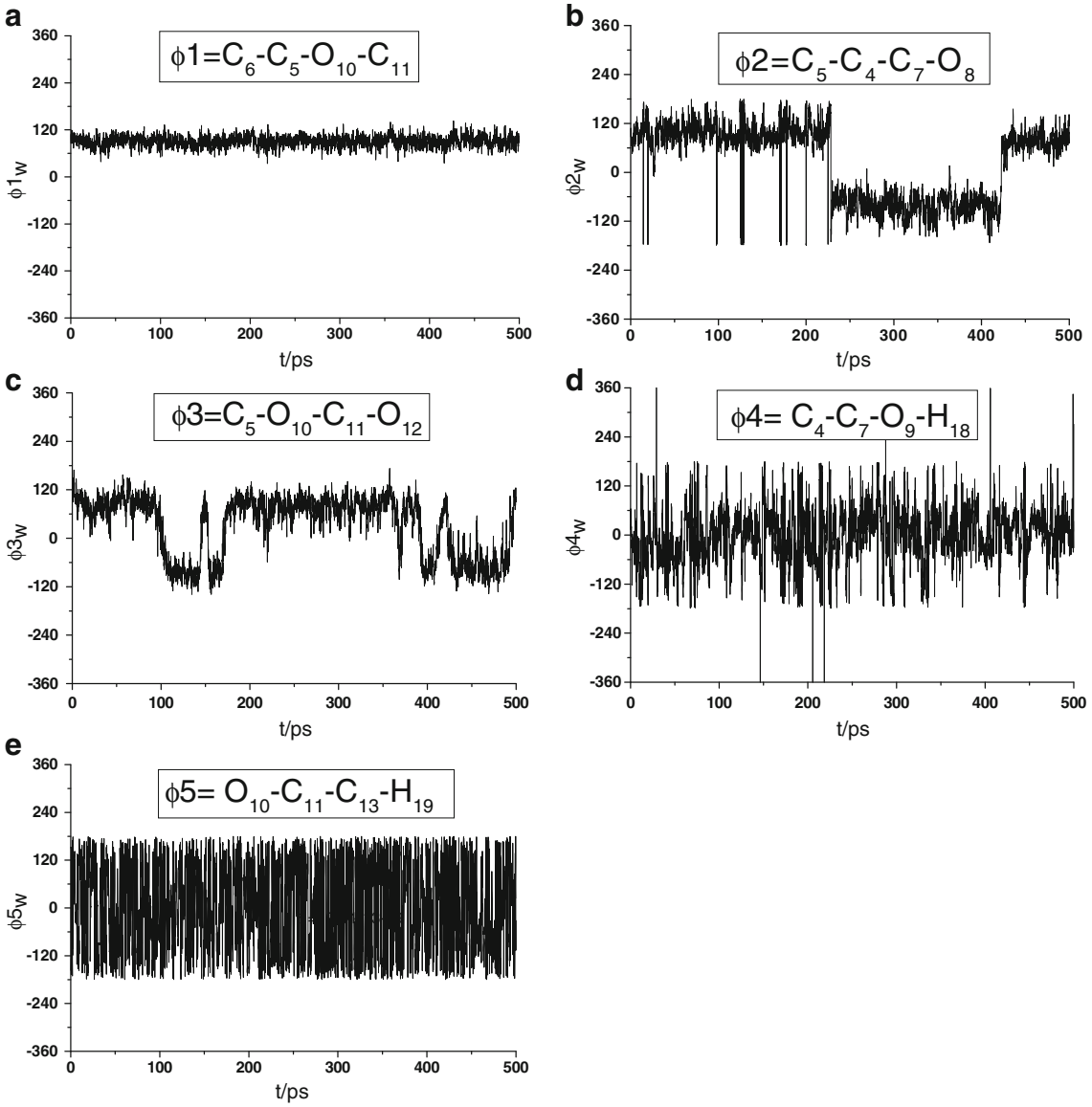

Fig. 3a–e Temporal trajectories evolution in aqueous solution (W). *x-axis* Time (ps), *y-axis* dihedral angles Φi, with i=1–5 (**a** Φ1, **b** Φ2, **c** Φ3, **d** Φ4 and **e** Φ5)

Table 1 Internal hydrogen bonds (HB). Criteria for HB [13]: distance between the proton and acceptor (d<0.24 nm), angle between donor–H–acceptor , (Φ>135°). *rms,Φ* Fluctuations of the Φ angle, *T/2* mean life time of the HB, *occur* occurrence over the 5,000 total configurations, *P* time ratio (%) over which HB exists

H	Structure bond	<Φ/o>	rms,Φ	<d/nm>	occur	P(%)	T/2
H_{18}	**1**. O_9-H_{18}–O_{12}	152.7	0.019	0.197	4850	97	211
	2. O_9-H_{18}–O_{10}	140.5	0.002	0.192	31	0.6	3
	3. O_9-H_{18}–C_{11}	152.3	0.000	0.232	30	0.6	2
H_{19}	**4**. CH_{13}-H_{19}–O_8	145.9	1.473	0.220	40	0.8	3
	5. CH_{13}-H_{19}–O_9	147.6	3.693	0.217	15	0.3	5
H_{20}	**6**. CH_{13}-H_{20}–O_8	147.5	1.475	0.217	33	0.7	2
	7. CH_{13}-H_{20}–O_9	137.1	3.639	0.212	14	0.1	0
H_{21}	**8**. CH_{13}-H_{20}–O_8	143.4	1.473	0.217	23	0.5	3
	9. CH_{13}-H_{21}–O_9	143.4	3.639	0.212	9	0.2	2

arrangements give rise to nine probable structures (Table 1). HB are a dynamic property; a HB can break temporarily due to a fluctuation that has no rearrangement value, i.e., the HB will reform, and the network will relax locally to the same state as before the fluctuation occurred [14, 15]. The mean lifetimes (T/2) of HB [16] were calculated as the rate of the total time for which the HB was found to be connected. The duration of one connection is determined as the number of consecutive frames ("occurrence", defined as "occur" in Table 1) in which the HB was found to be connected, multiplied by the simulation time step. To analyze our results, we defined P as a measure of the persistence of the HB; as such, P is the ratio (%) of the time the HB was found to be connected to the total simulation time.

The strongest interaction observed is the HB formed between the hydroxyl H_{18} hydrogen, and the carboxylic O_{12} oxygen (structure 1, isomer O_9–H_{18}–O_{12}). It must be noted that this hydrogen is likely to form a steady bond since P had a value of 97 % and a mean lifetime of 211 ps. The long angle (Φ=152.7°) and the adequate distance (d= 0.197 nm) favors the interaction. This type of HB between two neighboring lateral groups results in the formation of an eight-membered ring. The length ("d" in Table 1) of the bond H_{18}–O_{12} is 0.197 nm, (Fig. 4). This particular arrangement is a closed aspirin conformer, with the eighth side being the hydrogen bond (H_{18}–O_{12}). Another HB interaction is observed between the lateral groups, with less probability of occurrence (0.6%), but favored by the adequate distance (d=0.192 nm). This weak HB is formed between the hydroxyl H_{18} atom, and the phenolic O_{10} oxygen (structure 2, isomer O_9–H_{18}–O_{10}). This HB results in the formation of a six-membered ring, and the length of the bond H_{18}–O_{10} is 0.192 nm. This is another likely closed aspirin conformational isomer.

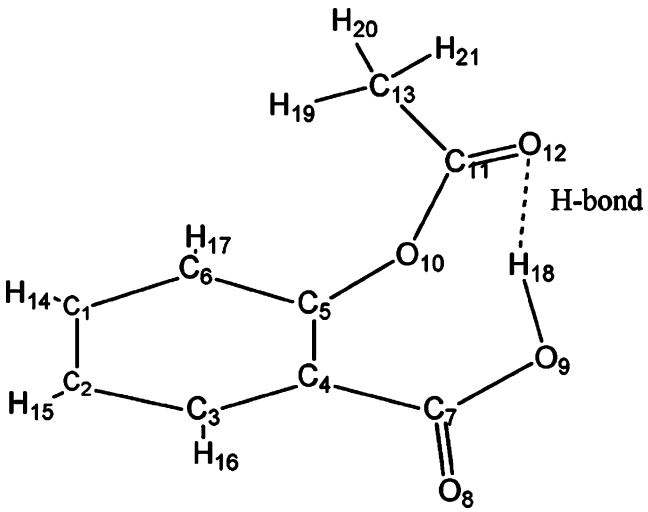

Fig. 4 Structure 1: eight-membered ring. The most probable structure of aspirin in water

Intramolecular HB have been found in aspirin by ab initio studies by Glaser [7], working at B3LYP/6-31 G(d) level of calculation, and in a more complete study by Ouvrard and Price [8] based on the analysis of aspirin gas phase conformers and multiple searches for minima in the lattice energy. Based on the current study, the work by Glaser [7] is a particular case of these previously cited papers. Our current results in vacuum agree with those of Glaser, while the results in aqueous solution are in concordance with those of Ouvrard and Price [8]. As Price pointed out [17], "for many simple molecules the forces between the molecules are weaker than the forces that define their covalent bonds, that we expect the molecular structure to be the same in the gas phase and in all solid form. In this case it is possible to determine the molecular structure by ab-initio calculations on the isolated molecule, assuming that this will be the molecular structure in the crystalline form". From our results, as assessed here, simulating a system in vacuum ([9], Chap. 2.8.2, p II-99) corresponds to the gas phase at zero pressure.

Many pharmaceutical drugs are conformationally mobile molecules and the action of these drugs is often determined by their conformation in solution. Our MD simulation revealed that the current structure (**1**; Table 1) is the most probable structure for aspirin in aqueous solution, since it involves the longest-lived HB. This particular arrangement is a closed aspirin conformer (Fig. 4).

Conclusions

The results of MD simulation show that aspirin has a relative flexible structure in aqueous solution, whereas the acetyl and carboxylic acid groups have restricted movements. The statistics reveal the existence of some dynamic intramolecular HB between these neighboring groups, in agreement with the ab initio evidence. There is also a striking HB interaction (structure 1, isomer O_9–H_{18}–O_{12}) formed between the hydroxyl H_{18} hydrogen and the carboxylic O_{12} oxygen. This HB pattern forces the molecule to adopt a closed conformation resembling a closed eight-membered ring, during almost the entire simulation (97%), and, as in consequence, this is the most probable structure in water solution. Another HB interaction (structure 2, O_9–H_{18}–O_{12}) leads aspirin to a closed six-membered ring conformation (with lesser probability, P= 0.6%). These internal HB inhibit the rotational movements of the neighboring groups (fixing their orientation), maximizing their repulsive interaction and playing an important role in the stabilization of their conformation in aqueous solution.

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