Gas-Phase Conformations

DOI: 10.1002/anie.201106621

Unveiling the Shape of Aspirin in the Gas Phase**

Carlos Cabezas, José L. Alonso, Juan C. López, and Santiago Mata*

Aspirin (acetylsalicylic acid) is regarded as one of the oldest and more widely known drugs all over the world.[1] Aspirin has been used for a long time as analgesic, antithrombotic, antipyretic, and antirheumatic, but during the last decades, additional uses such as its administration on Alzheimer patients,[2] derived from its anti-inflammatory power, have been added to the more prominent ones. To date, its structure has only been studied in crystals by both X-ray[3] and neutron diffraction.[4] The polymorphism of aspirin is still an enigma despite numerous experimental studies,[5] and its structure in the gas phase is unknown. Up to nine conformers have been postulated by ab initio calculations but no direct evidence of their existence has been found.[6] The lack of experimental studies on aspirin in the gas phase, where it adopts a neutral form, is most likely because of the fact that it is a solid with a high melting point (m.p. 134–136 °C). In the last years laser ablation molecular beam Fourier transform microwave (LA-MB-FTMW) spectroscopy[7] has emerged as a definitive gas-phase structural probe of solid biomolecules with high melting points, such as amino acids[8] or nucleic acid bases,[9] providing accurate structural information because of individual conformers. Here we report the first rotational study of supersonically cooled aspirin using this technique together with its conformational analysis. Solid rods of aspirin were vaporized by the third harmonic of a Nd:YAG laser and the neutral ablation products were expanded supersonically to form a molecular beam where the aspirin molecules are probed with microwave radiation.

Starting with the rotational constants predicted by ab initio calculations[10] for the nine lower-energy conformers (Table S1 in the Supporting Information), wide frequency scans were conducted to search for spectral signatures of aspirin. After extensive trial and error we were able to recognize eight sets of μ_c-type R-branch lines corresponding to two different rotamers labeled I and II. In addition to the Doppler doublets, since the expansion is parallel to the optical axis of the resonator, splittings of several MHz were observed in all the transitions (an illustration is given in Figure 1). Given that aspirin is a closed shell molecule no other hyperfine structure in the rotational spectra except that arising from the coupling between the internal and overall rotation is expected to occur. Thus, we attributed the splitting shown in Figure 1 to the internal rotation of the methyl group causing the occurrence of the *A*–*E* doublets.[11]

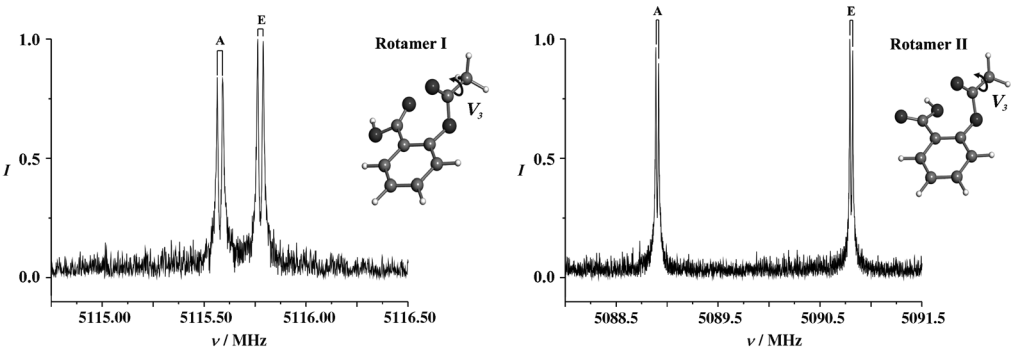


Figure 1. The $3_{1,2} \leftarrow 2_{0,2}$ rotational transition for both aspirin species showing the *A*–*E* doublets because of the internal rotation of the methyl group. Since the molecular beam and the microwave radiation travel parallel to each other in our setup, each component appears splitted because of the Doppler effect.

The measured transitions for the *A*-torsional state were analyzed using the Watson[12,13] semirigid rotor Hamiltonian ($H_R^{(A)}$) in the asymmetric (*A*) reduction. The fits provided an initial set of rotational constants that allowed the prediction and measurement of a new group of transitions belonging to the μ_a- and μ_b-types for rotamers I and II, respectively. The final analysis of the complete sets of measured transitions permitted us to determine the rotational constants of both rotamers listed in Table 1. All measured frequencies along with a complete set of spectroscopic parameters including centrifugal distortion constants are provided in Tables S2–S4 in the Supporting Information.

Aspirin may exist in various forms differing from each other by the arrangements of the carboxylic and ester groups

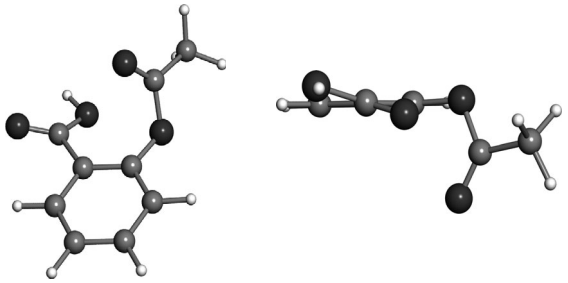
[*] Dr. C. Cabezas, Prof. J. L. Alonso, Prof. J. C. López, S. Mata
Grupo de Espectroscopía Molecular (GEM)
Edificio Quifima, Laboratorios de Espectroscopia
y Bioespectroscopia, Parque Científico UVa
Universidad de Valladolid, 47011 Valladolid (Spain)
E-mail: jlalonso@qf.uva.es
Homepage: http://www.gem.uva.es

[**] This research was supported by the Ministerio de Ciencia e Innovación (grant numbers CTQ 2006-05981/BQU, CTQ 2010-17436, and Consolider Ingenio 2010 CSD 2009-00038), Junta de Castilla y León (grant number VA070A08). Carlos Cabezas thanks the Ministerio de Ciencia e Innovación for a FPI grant.

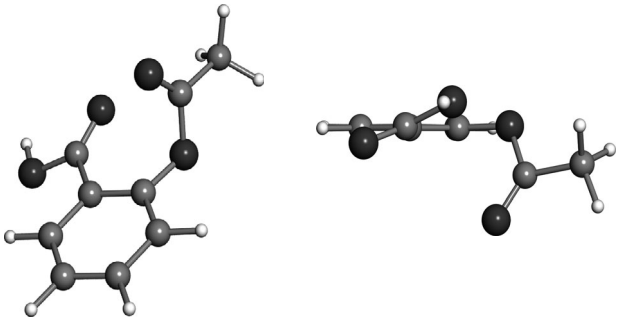
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201106621.

Table 1: Rotational parameters for the lowest-energy conformers and the two identified forms of aspirin (top: conformer 1a; bottom: conformer 2a).

Parameter[a]	Theoretical values Conformer 1a[c]	Experimental value Rotamer I[d]
A [MHz]	1136	1156.06755 (32)[e]
B [MHz]	766	762.640100 (80)
C [MHz]	506	508.955730 (60)
$\lvert\mu_a\rvert$ [D][b]	1.3	in agreement with theory
$\lvert\mu_b\rvert$ [D]	0.1	
$\lvert\mu_c\rvert$ [D]	1.2	in agreement with theory



	Conformer 2a	Rotamer II
A [MHz]	1176	1155.20533 (37)
B [MHz]	754	758.10774 (10)
C [MHz]	518	511.420721 (75)
$\lvert\mu_a\rvert$ [D][c]	0.4	
$\lvert\mu_b\rvert$ [D]	1.9	in agreement with theory
$\lvert\mu_c\rvert$ [D]	1.9	in agreement with theory



[a] *A*, *B*, and *C* are the rotational constants, μ_a, μ_b, and μ_c are the electric dipole moment components. [b] 1 D $\approx 3.3356\times10^{-30}$ C m. [c] Optimized structures at the MP2/6-311++G(d,p) level of calculation (taken from Table S1 in the Supporting Information). The Cartesian coordinates of all atoms in the principal inertial axis system for the two lowest-energy conformers are given in Table S7 in the Supporting Information. [d] The values of the experimental rotational parameters were extracted from the fit using a Watson *A*-reduced semirigid rotor Hamiltonian; $(H_R^{(A)}) = A P_a^2 + B P_b^2 + C P_c^2 - \Delta_J P^4 - \Delta_{JK} P^2 P_a^2 - \Delta_K P_a^4 - 2\delta_J P^2 (P_b^2 - P_c^2) - \delta_K [P_a^2 (P_b^2 - P_c^2) + (P_b^2 - P_c^2) P_a^2]$, where *A*, *B*, and *C* are the rotational constants and Δ_J, Δ_{JK}, Δ_K, δ_J, δ_K are the quartic centrifugal distortion constants. [e] The standard errors are given in parentheses in units of the last digit.

(see Table S1 in the Supporting Information). The primary basis for the identification of the detected rotamers was the comparison of the experimental values of the rotational constants of Table 1 with those predicted for the nine conformers postulated ab initio (see Table S1 in the Supporting Information). The experimental values are in good agreement with those predicted for conformers 1a and 2a (also collected in Table 1 for comparison). The fact that no μ_b-type transitions were detected for rotamer I is consistent with the very low predicted value of the μ_b dipole moment for conformer 1a ($\mu_b = 0.1$D). In addition, no μ_a-type transitions were observed for I which is consistent with a low value of μ_a dipole moment component as predicted for conformer 2a ($\mu_a = 0.4$ D). Additionally, the microwave power necessary for the optimal polarization of the transitions of rotamers I and II is in agreement with the predicted dipole moment components of conformers 1a and 2a, respectively. With all this information we identified rotamer I as conformer 1a and rotamer II as conformer 2a. Both structures of aspirin are rotational isomers differing in the position of the hydroxyl group with respect to the ester group. The relative intensity measurements performed on rotational transitions provide an estimation of their populations in the supersonic expansion. The experimental ratio (1a/2a = 4:1) is in accordance with the computed energies that predict conformer 2a about 275 cm^{-1} above the global minimum (1a). Although considerable efforts were made to detect other conformers of aspirin no new sets of transitions were discovered. This can be attributed to the much lower abundance (higher relative energy) of these conformers in the supersonic expansion relative to the two conformers observed in this study.

The internal rotation barrier V_3 for both conformers has been determined using the internal axis method in the form given by Woods.[14,15] The *A*–*E* splittings (see Tables S5–S6 in the Supporting Information) because of the coupling of internal and overall rotation have been used to determine the internal rotation parameters summarized in Table 2. The experimental values of the rotational barriers of rotamers I

Table 2: Methyl internal rotation experimental parameters for the two identified forms of aspirin.

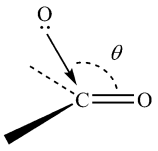
Parameter	Rotamer I	Rotamer II
V_3 [cm^{-1}][a]	244.26 (23)[e]/300.79[f]	191.38 (14)/269.31[g]
∡(i,a) [°][b]	21.6262 (63)	21.1003 (24)
∡(i,b) [°]	92.4822 (6)	83.0097 (8)
∡(i,c) [°]	68.5311 (56)	70.1958 (22)
$\nu_{torsion}$[c]	107.823 (50)	95.796 (36)
s[d]	20.530 (19)	15.964 (12)

[a] V_3 is the internal rotation barrier. [b] Angles between the top rotational axe and the principal axes system. [c] Torsional frequency calculated using the high-barrier approximation: $\nu = 3(V_3 F)^{1/2}$. [d] Reduced barrier. [e] Standard error in parentheses and in units of the last digit. [f,g] Theoretical values of the V_3 rotation barrier for conformers 1a and 2a, respectively.

and II are also consistent with those predicted theoretically for conformers 1a and 2a, respectively (see Table 2). The observed values are higher than those observed for methyl, ethyl, isopropenyl, and allyl acetates were the observed barriers were of 99.6, 101.6, 135.4, and 98.1 cm^{-1}.[16] The differences can be explained taking into account how the environment of the methyl group changes in aspirin with respect to the aforementioned acetates.

Although the data available do not allow a detailed investigation, simple considerations based on the experimental observations may lead to important conclusions about the planarity of the observed conformers of aspirin. The planar moment of inertia $P_{cc} = (I_a + I_b - I_c)/2 = \Sigma_i m_i c_i^2$, where m_i and c_i are, respectively, the mass and c coordinate of atom i, gives the mass extension out from the *ab* inertial plane. For example, salicylic[17] and benzoic acids[18] were reported to be planar and show P_{cc} values of 0.127 and 0.183 u$Å^2$, respectively. For a planar skeleton of aspirin only the methylic hydrogen atoms would contribute to the planar moment as in methylsalicylate[19] and this would give a value of P_{cc} close to $I_{CH_3}/2$ as determined from the internal rotation analysis. The experimental values of the planar moments (about −53 and −58 u$Å^2$, see Table S2 in the Supporting Information) left no doubt that the skeleton of aspirin is nonplanar in both observed conformers. Given the good agreement between the observed and predicted data one can assume that the actual structures of the observed conformers of aspirin are close to those predicted by ab initio calculations. In these structures the benzene ring does not maintain coplanarity neither with the acid group nor with the acetate group. The origin of this non-planarity of the skeleton of aspirin has been attributed on the basis of theoretical calculations to steric repulsions.[6]

The structure of conformer 1a of aspirin shows that the carboxyl oxygen approaches to the ester carbonyl group in a way that resembles the Bürgi–Dunitz trajectory[20] which describes the most favorable approach of a nucleophile (O:) to a carbon of a carbonyl group in an addition reaction. The same arrangement exists in conformer 2a between the carbonyl oxygen of the ester group and the carboxy carbon. The calculated Bürgi–Dunitz parameters are $R_{OC} =$ 2.75 Å, $\theta = 95.2°$ for conformer 1a and $R_{OC} = 2.79$ Å, $\theta = 91.4°$ for conformer 2a. This arrangement is usually related to the existence of n→π* interactions which imply the delocalization of an electron pair of the nucleophile (O:) into the antibonding π* orbital of the carbonyl group. We have performed an NBO analysis[21] of these forms of aspirin at the B3LYP/6-311++G(d,p) level that further supports the existence of this n→π* interaction. The calculations yield stabilization energies of 12.4 kJ mol^{-1} for conformer 1a and 8.7 kJ mol^{-1} for conformer 2a. We have observed this kind of interaction in biomolecules such as 4(*S*)-hydroxyproline,[22] γ-aminobutyric acid,[23] and β-alanine.[24] In a theoretical work just published on aspirin, the importance of this interaction has been widely analysed.[25]



In summary, we report the first rotational spectra of aspirin and conclude that aspirin is present in two dominant conformers in the gas phase (Table 1). The experimental results may serve as an excellent point of reference for further structure elucidation of other related solid drugs.

Experimental Section

The rotational spectrum of aspirin was investigated by using a LA-MB-FTMW spectrometer[7] which works in the 3–12 GHz frequency region. Solid rod samples were made from powdered aspirin (99%, Aldrich) and vaporized by ablation with the third harmonics of a picoseconds Nd:YAG laser (about 15 mJ per pulse, 35 ps width pulse). The vaporized molecules were seeded in the carrier gas (Ne: 15 bar) and expanded supersonically between the mirrors of the evacuated Fabry–Pérot microwave resonator creating a molecular beam. A microwave radiation pulse (0.3 ms), in the operating frequency range of the spectrometer, was subsequently applied to cause the macroscopic polarization of the molecules in the beam. The immediate molecular de-excitation signal, which contains the resonance frequencies corresponding to the rotational transitions, was collected and transformed to the frequency domain spectrum by a Fourier transform process. The spectrometer has a collinear disposition between the supersonic jet and the microwave resonator axis, for this reason each line in the spectrum appeared as a Doppler doublet, and the transition frequencies are calculated as the arithmetic mean of the Doppler components. Different experiments, at the same frequency polarization, can be phase coherently co-added, so thousand cycles can be made for the measurements of very weak transitions. The estimated accuracy of the frequency measurements is better than 3 kHz.

Received: September 18, 2011
Published online: December 28, 2011

Keywords: aspirin · conformational analysis · laser ablation · microwave spectroscopy · supersonic jets

[1] J. R. Vane, R. M. Bottling, *Aspirin and Other Salicylates*, Chapman and Hall, London, **1992**.
[2] a) J. Schnabel, *Science* **1993**, *260*, 1719–1720; b) E. Pennisi, *Science* **1998**, *280*, 1191–1192.
[3] a) P. J. Wheatley, *J. Chem. Soc.* **1964**, 6036; b) Y. Kim, K. Machida, T. Taga, K. Osaki, *Chem. Pharm. Bull.* **1985**, *33*, 2641.
[4] C. C. Wilson, *New J. Chem.* **2002**, *26*, 1733.
[5] a) P. Vishweshwar, J. A. McMahon, M. Oliveira, M. L. Peterson, M. J. Zaworotko, *J. Am. Chem. Soc.* **2005**, *127*, 16802; b) A. D. Bond, R. Boese, G. R. Desiraju, *Angew. Chem.* **2007**, *119*, 625–630; *Angew. Chem. Int. Ed.* **2007**, *46*, 618–622.
[6] R. J. Glaser, *J. Org. Chem.* **2001**, *66*, 771–779.
[7] a) S. Antolínez, A. Lesarri, S. Mata, S. Blanco, J. C. López, J. L. Alonso, *J. Mol. Struct.* **2002**, *612*, 125; b) A. Lesarri, S. Mata, J. C. López, J. L. Alonso, *Rev. Sci. Instrum.* **2003**, *74*, 4799–4804; c) A. Lesarri, S. Mata, S. Blanco, J. C. López, J. L. Alonso, *J. Chem. Phys.* **2004**, *120*, 6191.
[8] J. L. Alonso, C. Pérez, M. E. Sanz, J. C. López, S. Blanco, *Phys. Chem. Chem. Phys.* **2009**, *11*, 617–627.
[9] a) V. Vaquero, M. E. Sanz, J. C. López, J. L. Alonso, *J. Phys. Chem. A* **2007**, *111*, 3443; b) J. C. López, M. I. Peña, M. E. Sanz, J. L. Alonso, *J. Chem. Phys.* **2007**, *126*, 191103; c) J. L. Alonso, M. I. Peña, J. C. López, V. Vaquero, *Angew. Chem.* **2009**, *121*, 6257; *Angew. Chem. Int. Ed.* **2009**, *48*, 6141.
[10] M. J. Frisch, et al., Gaussian 03, Revision B.04, Gaussian, Inc., Pittsburgh PA, 2003; see the Supporting Information.
[11] W. Gordy, R. L. Cook, *Microwave Molecular Spectra*, Wiley-Interscience, New York, **1984**, chap. IX and XIV.
[12] J. K. G. Watson in *Vibrational Spectra and Structure*, *Vol. 6* (Ed.: J. R. Durig), Elsevier, Amsterdam, **1977**, pp. 1–89.

[13] H. M. Pickett, *J. Mol. Spectrosc.* **1991**, *148*, 371 – 377.
[14] H. Hartwig, H. Dreizler, *Z. Naturforsch. A* **1996**, *51*, 923 – 932.
[15] a) R. C. Woods, *J. Mol. Spectrosc.* **1966**, *21*, 4; b) R. C. Woods, *J. Mol. Spectrosc.* **1967**, *22*, 49.
[16] a) J. Sheridan, W. Bossert, A. Bauder, *J. Mol. Spectrosc.* **1980**, *80*, 1; b) D. Jelisavac, D. Cortés Gómez, H. V. L. Nguyen, L. W. Sutikdja, W. Stahl, I. Kleiner, *J. Mol. Spectrosc.* **2009**, *257*, 111; c) H. V. L. Nguyen, W. Stahl, *J. Mol. Spectrosc.* **2010**, *264*, 120 – 124; d) H. V. L. Nguyen, H. Mouhib, W. Stahl, I. Kleiner, *Mol. Phys.* **2010**, *108*, 763.
[17] L. Evangelisti, S. Tang, B. Velino, W. Caminati, *J. Mol. Struct.* **2009**, *921*, 285.
[18] M. Onda, M. Asai, K. Takise, K. Kuwae, K. Hayami, A. Kuroe, M. Mori, H. Miyazaki, N. Suzuki, I. Yamaguchi, *J. Mol. Struct.* **1999**, *482*, 301.
[19] S. Melandri, B. M. Giuliano, A. Maris, L. B. Favero, P. Ottaviani, B. Velino, W. Caminati, *J. Phys. Chem. A* **2007**, *111*, 9076.
[20] a) H. B. Bürgi, J. D. Dunitz, E. Shefter, *J. Am. Chem. Soc.* **1973**, *95*, 5065 – 5067; b) H. B. Bürgi, J. D. Dunitz, J. M. Lehn, G. Wipff, *Tetrahedron* **1974**, *30*, 1563 – 1572; c) H. B. Bürgi, J. D. Dunitz, *Acc. Chem. Res.* **1983**, *16*, 153 – 161.
[21] A. E. Reed, L. A. Curtiss, F. Weinhold, *Chem. Rev.* **1988**, *88*, 899 – 926.
[22] A. Lesarri, E. J. Cocinero, J. C. López, J. L. Alonso, *J. Am. Chem. Soc.* **2005**, *127*, 2572 – 2577.
[23] S. Blanco, J. C. López, S. Mata, J. L. Alonso, *Angew. Chem.* **2010**, *122*, 9373; *Angew. Chem. Int. Ed.* **2010**, *49*, 9187.
[24] M. E. Sanz, A. Lesarri, M. I. Peña, V. Vaquero, V. Cortijo, J. C. López, J. L. Alonso, *J. Am. Chem. Soc.* **2006**, *128*, 3812 – 3817.
[25] A. Choundary, K. J. Kramer, R. Raines, *J. Org. Chem.* **2011**, DOI: 10.1021/jo201389d.

