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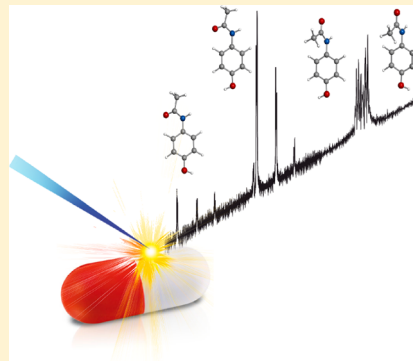
Rotational Spectrum of Paracetamol

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S Supporting Information

ABSTRACT: In this paper we report the first rotational spectrum of isolated paracetamol. Solid samples of paracetamol were vaporized by laser ablation, expanded in a supersonic jet and characterized by Fourier transform microwave spectroscopy in the 4–10 GHz frequency range. The spectra of a total of four conformers, two with a trans peptidic –CO–NH– functional group arrangement and two with a cis peptidic group arrangement have been observed for the first time. The identification of all the conformers was based on the comparison of the experimental rotational and ^{14}N quadrupole coupling constants with those predicted by ab initio calculations, and relative values of their electrical dipole moment components.



■ INTRODUCTION

Acetaminophen, also known as paracetamol (from *para*-(acetyl-amino)phenol) is an analgesic–antipyretic agent used as an alternative to aspirin, although its anti-inflammatory effects are much weaker than the latter. Acetaminophen was first used in medicine in 1893, but only after 1949 has it gained popularity, when it was recognized as the major active metabolite of both acetanilide and phenacetin.¹ Chemically, paracetamol is very similar to acetanilide, from which it can be derived by including an OH group at the phenyl ring in the para position. Given the possible trans and cis arrangements of the peptidic –CO–NH– functional group and the relative orientation of the OH group in the para position, the four conformers of paracetamol shown in Figure 1 should be considered. Because this molecule possesses a chromophore group, it has been recently studied by electronic spectroscopy techniques in supersonic expansions. In 2010, Beames and Hudson² reported the first electronic spectrum of paracetamol and its HB spectra in the gas phase showed the existence of only one conformer. Lee et al.³ in 2011 distinguished two different conformers by UV–UV HB spectroscopy and very recently Sohn et al.⁴ using IR and UV double resonance spectroscopy identified four different species belonging to trans conformers. Two of these species arise from vibrationally excited levels of the electronic ground state, associated to the methyl group torsion. All these results are in contrast with previous observations for the related molecules of formanilide and acetanilide, for which both trans and cis arrangements of the peptidic group were detected.^{5,6} To date, no evidence of cis conformers for paracetamol has been yet reported.

Laser ablation, in combination with Fourier transform microwave spectroscopy,⁷ has proven to be a powerful tool in the investigation of the gas phase conformational behavior of high melting point molecules like amino acids,^{8,9} nucleic acid

bases,¹⁰ or neurotransmitters.¹¹ It has been very recently employed to unveil the conformational behavior of aspirin.¹² In the experimental procedure, solid rods are vaporized by laser ablation and the neutral products expanded supersonically to form a molecular beam. In such an environment the conformers that kept enough population can be probed by Fourier transform microwave spectroscopy. On this basis, we present here the first rotational study of paracetamol using LA-MB-FTMW spectroscopy.

■ EXPERIMENTAL AND COMPUTATIONAL METHODS

Experimental Setup. A laser ablation molecular beam Fourier transform microwave (LA-MB-FTMW) spectrometer, described elsewhere,^{7–12} which operates in the 4–10 GHz frequency region, was used to record the rotational spectra of paracetamol. Solid fine-powdered paracetamol (Sigma-Aldrich, mp = 167 °C) was mixed with minimum quantities of a commercial binder (methyl cellulose) to form a cylindrical rod. The samples were then vaporized using the third harmonic (355 nm) of a Nd:YAG picosecond laser (20 ps length pulse) using energies of ~13 mJ/pulse. The neutral vaporized molecules were seeded in the carrier gas (Ne at 15 bar and room temperature, nozzle diameter = 1 mm) and expanded into a Fabry–Pérot resonator. After sending the microwaves pulses through the cavity, the emission free induction decay (FID) of the molecules was recorded in the time-domain and Fourier transformed to yield the frequency-domain spectrum.

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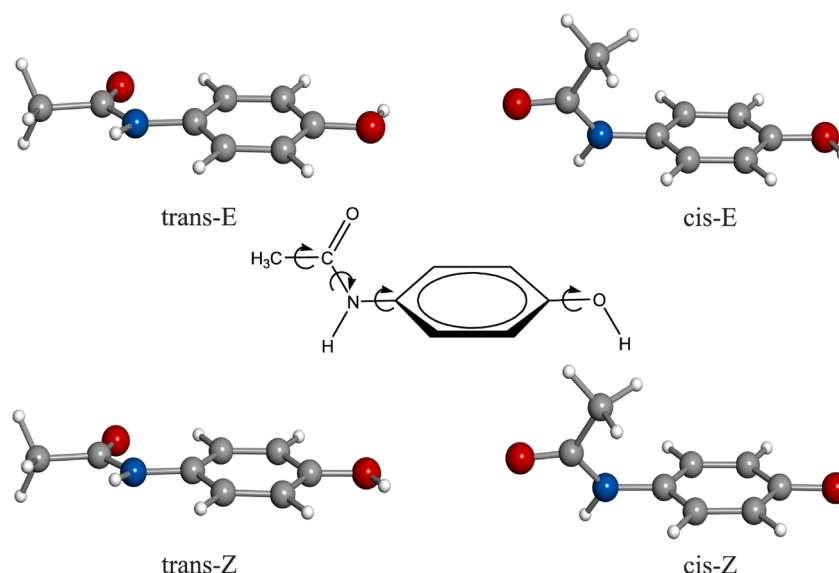


Figure 1. Four plausible conformers of paracetamol. To label the conformers, we have used the same notation employed in the previous studies of refs 4 and 6. An additional index is needed to denote the relative arrangements of the OH groups, zusammen (Z) or entgegen (E), with respect to the peptidic group.

Table 1. Calculated and Experimental Spectroscopic Parameters for the Plausible Conformers and for the Observed Rotamers of Paracetamol

	theory ^a				experimental			
	trans-E	trans-Z	cis-E	cis-Z	rotamer I	rotamer II	rotamer III	rotamer IV
A^b	3568.5	3557.4	3273.3	3270.6	3668.26678 (83) ^h	3664.8110 (17)	<i>i</i>	<i>i</i>
B	546.2	546.7	554.6	554.8	546.763941 (89)	546.9009 (16)	547.9993 (78)	547.9572 (58)
C	481.0	483.0	520.2	520.7	476.536462 (62)	476.61660 (51)	530.9044 (78)	530.9063 (57)
χ_{aa}	2.24	2.26	2.68	2.78	2.1127 (36)	2.1176 (36)	<i>i</i>	<i>i</i>
χ_{bb}	1.48	1.42	−1.32	−1.43	1.7149 (44)	1.7188 (43)	<i>i</i>	<i>i</i>
χ_{cc}	−3.72	−3.68	−1.36	−1.26	−3.8276 (44)	−3.8364 (43)	<i>i</i>	<i>i</i>
$ \mu_a $	1.0	0.1	4.1	4.4				
$ \mu_b $	2.4	4.6	1.8	0.9				
$ \mu_c $	1.3	0.6	0.1	1.1				
Δ_c	−16.2	−20.1	−94.1	−94.9	−1.55446 (32)	−1.6312 (39)		
ΔE^c	0	125	281	285				
ΔG^{cd}	0	109	363	345				
V_3^e	88	76	56	48				
N^f					59	22	8	8
σ^g					2.6	2.4	75	55

^aAb initio computations at MP2/6-311++G(d,p) level of theory. ^b A , B , and C represent the rotational constants in MHz; χ_{aa} , χ_{bb} , and χ_{cc} are the diagonal elements of the ¹⁴N nuclear quadrupole coupling tensor in MHz; μ_a , μ_b , and μ_c are the electric dipole moment components in D; $\Delta_c = I_c - I_a - I_b$ is the inertial defect in u Å². ^cRelative energies with respect to the global minimum in cm^{−1} calculated at MP2/6-311++G(d,p) level of theory. ^dGibbs energies calculated at 298 K at the MP2/6-311++G(d,p) level of theory in cm^{−1}. ^e V_3 internal rotation barrier calculated at the MP2/6-311++G(d,p) level of theory in cm^{−1}. ^fNumber of fitted transitions. ^grms deviation of the fit. ^hStandard error in parentheses in units of the last digit. ⁱNot determined from available data.

Because the supersonic jet and the microwave resonator axis are collinearly placed, signals appeared split into Doppler doublets. The arithmetic mean of the doublets was taken as the final frequency. The estimated accuracy of the frequency measurements is better than 3 kHz.

Ab Initio Calculations. To guide the spectral searches, full geometry optimizations have been carried out on the four plausible conformers of paracetamol shown in Figure 1. Ab initio calculations were performed at the MP2/6-311++G(d,p) level of theory, using the Gaussian 09¹³ suite of programs. We estimated the values of the rotational and ¹⁴N nuclear quadrupole coupling constants, electric dipole moment

components, and the values for the V_3 internal rotation of the methyl group barriers, which are collected in Table 1. Attending to the very low V_3 values, large A–E splittings due to the internal rotation of the methyl group are expected. As with acetanilide⁶ before, the perturbed “E” substate lines will not be considered in the analysis of the rotational spectra of paracetamol.

RESULTS AND DISCUSSION

Wide frequency scans of the spectra were directed to search for μ_b -type R-branch transitions of the trans conformers on the basis of the predicted large value of the μ_b electric dipole

moment component for both conformers. Recorded spectra soon lead to identification of two sets of μ_b -type R-branch rotational transitions as belonging to two different rotamers I and II of paracetamol. The pattern of Figure 2 for the $3_{13} \leftarrow 2_{02}$

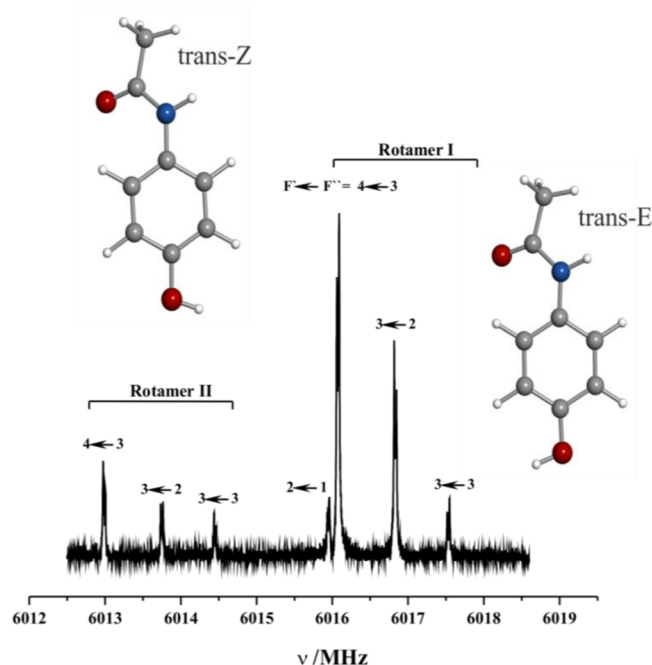


Figure 2. Section of the microwave spectrum of paracetamol (after 150 accumulation cycles) showing the $3_{13} \leftarrow 2_{02}$ rotational transition of rotamers I and II. Hyperfine components arising from the ^{14}N nuclear quadrupole coupling are labeled as $F' \leftarrow F''$ ($I + J = F$).

transition shows doubling very close in frequency which can be ascribed to the two different orientations of the OH group (Z or E configurations) in the trans conformers of paracetamol. Apart from the instrumental Doppler effects,⁷ all the measured transitions exhibited fully resolved ^{14}N nuclear quadrupole coupling hyperfine structure arising from interaction of the electric quadrupole moment of ^{14}N nucleus with the electric field gradient created at the site of this nucleus by the rest of the electrical charges of the molecule. The frequency measurements for rotamer I were extended to the μ_a -type R-branch transitions. Trials to observe the μ_a -type spectrum of rotamer II were unsuccessful. No lines obeying μ_c -type selection rules could be observed for either of the two rotamers, indicating a very small or zero electric dipole moment component along the c axis. All the measured hyperfine components collected in Tables S1 and S2 of Supporting Information were fitted¹⁴ using the Hamiltonian $\mathbf{H} = \mathbf{H}_R^{(A)} + \mathbf{H}_Q$, where $\mathbf{H}_R^{(A)}$ is the A-reduced rigid rotor Hamiltonian of Watson in the \mathbf{I}^r representation¹⁵ and \mathbf{H}_Q describes the nuclear quadrupole coupling interaction,¹⁶ which was set up in the coupled basis set (\mathbf{IJF}), $I + J = F$. The analysis rendered very precise values of rotational constants (A , B , and C) and diagonal elements of nuclear quadrupole coupling tensor (χ_{aa} , χ_{bb} , χ_{cc}) for rotamers I and II collected in Table 1.

Both sets of rotational and ^{14}N quadrupole coupling constants for rotamers I and II are similar and compatible with those predicted for the trans-E and trans-Z conformers. This reflects nearly the same mass distribution and orientation of the NH_2 group in the principal axis system in both conformers. Thus, the conformational assignment should be

based on the selection rules and the intensity of the observed rotational transitions.^{7,11} In this manner, the absence of μ_a -type spectrum and the low microwave power needed for optimal polarization of the μ_b -type transitions of rotamer II, consistent with the predicted values of the μ_a and μ_b dipole moment components for conformer trans-Z ($\mu_a = 0.1$ D and $\mu_b = 4.6$ D), clearly leads the identification of rotamer I as conformer trans-E. Rotamer II should be consistently identified as conformer trans-Z.

Inertial defect Δ_c values, which represent a measure of the nonplanarity of the molecular skeleton, should be zero for a strictly planar rigid molecule. As can be seen in Table 1, the theoretical values of Δ_c are far from zero (-16.2 and -20.1 u \AA^2 for trans-E and trans-Z species, respectively), suggesting that both conformers are predicted to have nonplanar skeletons. In contrast, the experimental values of Δ_c strongly differ from the predicted ones and are very close to zero (-1.55 and -1.63 u \AA^2 for trans-E and trans-Z species, respectively) and also close to -3.18 u \AA^2 , the calculated value for only two methyl hydrogen atoms out of the plane. Thus, it can be concluded that the conformers trans-Z and trans-E have a planar skeleton, with the methyl group hydrogen atoms as the sole out-of-plane mass contributors. This fact is further confirmed by the absence of μ_c -type transitions for any of two trans species, because the electric dipole moment component along the c axis must be zero for a planar skeleton.

Relative intensity measurements were done on different μ_b type transitions of the two trans conformers to estimate their relative abundances in the supersonic jet. The population ratio estimated, taking into account the predicted values of the dipole moment components of Table 1, is trans-E/trans-Z = 5/1 corresponding to a equilibrium conformational energy difference of about 300 cm^{-1} . The computed ΔG value (shown in Table 1) is 109 cm^{-1} . The same discrepancy in the ΔG values was observed in the previous study of acetanilide.⁶

Searches for cis forms of paracetamol were carefully performed in wide frequency regions, scouting for μ_a -type R-branch transitions, given that μ_a is the predominant dipole moment component for these species. Once all transitions belonging to the trans conformers were removed from the spectrum, several sets of μ_a -type R-branch lines, appearing as doublets of similar intensities, with not well resolved ^{14}N quadrupole pattern, were identified. Figure 3 illustrates the $S_{15} \leftarrow 4_{14}$ rotational transition. The center frequency of each doublet was separately

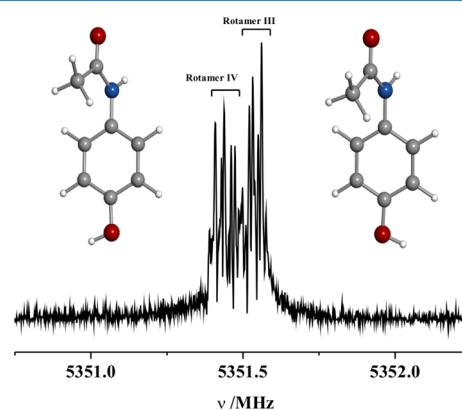


Figure 3. $S_{15} \leftarrow 4_{14}$ rotational transition for two cis conformers of paracetamol showing the very complicated ^{14}N nuclear quadrupole coupling hyperfine structure.

fitted,¹⁴ obtaining the rotational constants listed in Table 1, labeled as rotamers III and IV. Based upon the values of the *B* and *C* rotational constants, these doublets can be attributed to spectral signatures of both *cis-E* and *cis-Z* conformers of paracetamol, but with the available experimental data, it is not possible to discriminate between them.

Another possible explanation for the observed doublets could emerge from a tunneling motion in the *cis* conformers, as observed when acetanilide⁶ has been discarded. In paracetamol, the tunneling process presents a much more complicated pattern involving also the torsion of OH group with a very high barrier, which does not match with the spectroscopic observations.

CONCLUSIONS

The results presented in this paper detail the success of the LA-MB-FTMW technique in the study of drugs and unravelling high energy conformational species even in heavy and complex molecules such as paracetamol. In spite of its considerably higher energy with respect to the predominant *trans* conformers, we detected the rotational spectrum of the *cis* conformers of paracetamol. It should be noted that in previous studies only the *trans* forms have been observed in the gas phase.^{2–4} Hence this observation changes the previous vision of the conformational panorama of this drug and let us to conclude that the conformational behavior of paracetamol is similar to that of acetanilide,⁶ which exhibits both *trans* and *cis* conformers in the gas phase.

ASSOCIATED CONTENT

Supporting Information

Complete list of transition frequencies measured for the detected species of Paracetamol and complete ref 13. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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