

Identifying Enantiomers in Mixtures of Chiral Molecules with Broadband Microwave Spectroscopy**

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Abstract: Chirality-sensitive broadband microwave spectroscopy was performed on mixtures of carvone enantiomers and conformers to distinguish enantiomers, measure enantiomeric excesses, and determine the absolute configurations of the enantiomers. This method uses microwave three-wave mixing and is inherently well-suited to the analysis of mixtures—a unique advantage over other techniques. In contrast to conventional microwave spectroscopy, the phase of the received signal is also exploited. This phase depends upon the signs of the molecules' dipole-moment components and is used to identify the excess enantiomer. The measured signal amplitude determines the size of the excess. The broadband capabilities of the spectrometer were used to simultaneously excite and measure two conformers of carvone, demonstrating the analysis of a sample with multiple chiral species. Employing quantum chemical calculations and the measured phases, the absolute configurations of the enantiomers are determined.

Chirality plays a central role in many chemical and biological processes. A large fraction of biological molecules are chiral—the chemistry of life is built almost exclusively on left-handed amino acids and right-handed sugars, a phenomenon that is known as the “homochirality of life”. Despite the importance of chiral molecules, the experimental determination of enantiomeric excess (*ee*)—the fraction of left- versus right-handed molecules—within a mixture of chiral molecules remains a tremendous challenge. In this work, we demonstrate the clear differentiation between enantiomers within a mixture of chiral molecules using broadband rotational spectroscopy.^[1,2] In addition, we also measure the *ee* value of

mixtures and demonstrate how we can determine the absolute configuration of a chiral molecule.

Established spectroscopic methods to identify enantiomers and determine the *ee* value rely on the interaction of linearly or circularly polarized light with the target molecules. This interaction is relatively weak^[3] and thus highly concentrated samples or intense light fields are required. These methods, such as circular dichroism (CD), vibrational circular dichroism (VCD), and Raman optical activity (ROA) spectroscopy, are commercially available and widely used. However, they are less useful for the analysis of molecular mixtures, in particular when more than one chiral species is present. To the best of our knowledge, only one mixture analysis was reported so far, using Fourier transform VCD.^[4] Recent developments in this area employ synchrotron radiation and femtosecond lasers to study femtosecond optical activity, photoionization with subsequent fragment detection in coincidence, and circular dichroism in the photoelectron angular distributions (PAD) of chiral molecules.^[5–9]

Very recently, a new method based upon microwave spectroscopy was developed and demonstrated by us to differentiate enantiomeric pairs of chiral molecules in the gas phase.^[10] It was shown to be widely applicable to chiral molecules with dipole moment components $\mu_a, \mu_b, \mu_c \neq 0$. The underlying physical mechanism arises from the Hamiltonian of an asymmetric top in an external electric field. Ignoring small differences due to the parity-violating potential arising from the weak interaction, the rotational constants and dipole moment component magnitudes ($|\mu_a|$, $|\mu_b|$, and $|\mu_c|$) are equal for enantiomeric pairs of any chiral molecule and thus give rise to identical rotational spectra. All allowed rotational transitions can be classified as purely a-type, b-type, or c-type; that is, they exclusively depend on μ_a , μ_b , or μ_c , respectively. However, the product $\mu_a\mu_b\mu_c$ is of opposite sign for members of an enantiomeric pair since the dipole moments are mirrored. This fact is exploited in our method by using closed groups of transitions that include all three dipole-moment components (Figure 1A) in a three-wave mixing scheme, which can be viewed as a polarization-sensitive double-resonance experiment.^[11,12] In this way, a definitive chiral signature is obtained for nonracemic mixtures. For example, if we first interrogate μ_a and μ_c with two orthogonal fields (Figure 1A), the phases of the molecular signals in the third mutually orthogonal direction involving μ_b differ by π radians between enantiomers due to the change in sign of $\mu_a\mu_b\mu_c$ (see the Supporting Information). Furthermore, the signal amplitude is directly proportional to the *ee* value, as shown recently for a buffer-gas-cooled molecular sample.^[11] Sensitive measurements of enantiomeric excess are possible

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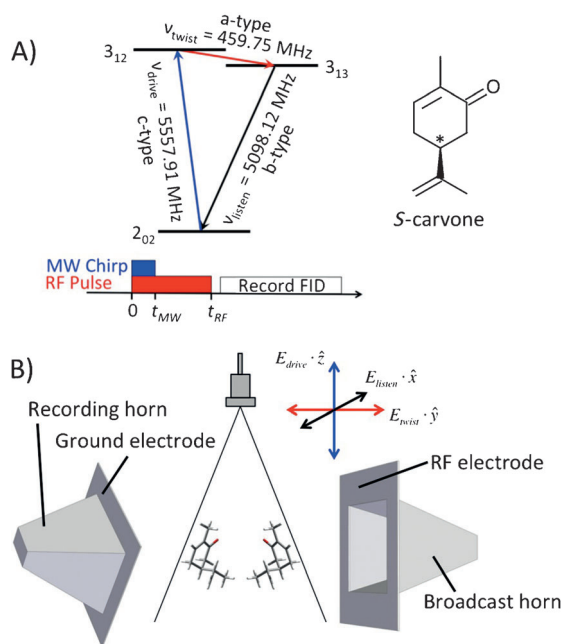


Figure 1. A) Energy level diagram and timeline for three-wave-mixing transitions of the carvone EQ2 conformation in which ν_{drive} , ν_{twist} , and ν_{listen} are the c-, a-, and b-type transition frequencies, respectively. B) Arrangement of the horn antennas with RF electrodes attached. The supersonic expansion is placed between the broadcast and receiving horns. The inset shows the polarizations of the excitation and emitted fields. Note that the broadcast horn is rotated 45° relative to the recording horn to allow for both conventional microwave spectroscopy and the detection of chiral signatures.

since three-wave mixing is strictly forbidden for a racemic mixture under any geometry in the electric dipole approximation, yielding an exactly defined zero background for a racemic sample.

In the present work, we extend this technique to measuring the individual chiral signatures, the *ee*, and the absolute configuration of a chiral molecule, all within a molecular mixture. We combine, for the first time, the techniques of microwave three-wave mixing and supersonic molecular jets as a source for internally cold molecules. Supersonic jets are widely used throughout the molecular spectroscopy community because they produce spectra that are greatly simplified relative to room-temperature measurements.

We use the terpene molecule carvone (Figure 1A) in this work. While *R*-carvone smells like spearmint, *S*-carvone smells like caraway, nicely demonstrating the different biological functionality of the enantiomers of chiral molecules. Under the cold conditions of a molecular jet, carvone exhibits two conformers, which we refer to as EQ1 and EQ2, following the nomenclature of Moreno et al.^[13]

We implemented chiral differentiation by introducing only minor modifications to the broadband chirped-pulse Fourier transform microwave (FTMW) spectrometer, COMPACT, which we operate in Hamburg.^[14] The molecule-microwave interaction region of the modified COMPACT spectrometer^[14] is shown schematically in Figure 1B, with details given in the Supporting Information. The microwave (MW) and radio-frequency (RF) fields were generated on the

same arbitrary waveform generator, thus giving us robust relative timing between the two pulses. We drive transitions among three energy levels connected by a-, b-, and c-type transitions (Figure 1A). Using the EQ2 conformer as an example, we resonantly drive a transition with a linearly polarized intense MW chirp (c-type, resonance at 5557.91 MHz), then “twist” the transition by means of a resonant RF pulse polarized orthogonal to the MW chirp (a-type, resonance at 459.75 MHz), and finally measure the phase-dependent free-induction decay (FID) signal that is polarized mutually orthogonal to both excitation pulses. Note that in this three-wave-mixing scheme, the “drive” and the “listen” frequencies differ by the RF “twist” frequency, so that a cavity spectrometer cannot be used because it does not allow for broadband operation.

Table 1 presents the independently measured three-wave-mixing results for the two conformers EQ2 and EQ1, that is, only a single conformation was excited during each measurement. The phase and amplitude of the listen signal represent the phase and amplitude of the Fourier coefficients at the listen frequency. The π radian phase difference between the enantiomers for a given phase of the RF pulse is robust. As a check, we shifted the phase of the RF radiation by π radians for both enantiomers (RF^-), which should result in a corresponding π radian phase-shifted signal. Furthermore, when the RF was turned off, no signal was observed at the target listen frequency.

Table 1: Phases (in radians) of enantiomerically pure *R*- and *S*-carvone from independent measurements of the two conformers, EQ1 and EQ2, with RF pulses, $\text{RF}^- = -(\text{RF}^+)$.

	Conformer	<i>S</i>	<i>R</i>	$\Delta\varphi^{[c]}$
RF^+	EQ1 ^[a]	−2.18(10)	0.83(21)	−3.01(23)
	EQ2 ^[b]	−1.37(14)	1.77(9)	−3.14(17)
RF^-	EQ1 ^[a]	0.92(20)	−2.06(18)	2.98(27)
	EQ2 ^[b]	1.81(9)	−1.36(11)	3.17(14)

[a] Drive chirp 3.0→2.9 GHz (0.250 μs), RF twist at 118.4 MHz (0.500 μs), listen at 2811.40 MHz. [b] Drive chirp 5.52→5.58 GHz (1 μs), RF twist at 459.75 MHz (5 μs), listen at 5098.12 MHz. [c] $\Delta\varphi$ describes the conformer-specific phase difference between the two enantiomers for one set of experiments.

It should be noted that the phase reported here depends upon the experimental conditions (e.g. MW and RF pulse durations, instrument-introduced delays, etc.) that define the beginning of FID recording. Thus, the reported phases are shifted from the absolute phases. Because different MW and RF pulses were used for each conformation, the phases were not shifted by the same amount for each conformation and thus will not necessarily have the same sign (see the Supporting Information for more details). It is the relative phase between the enantiomers that is the unambiguous signature that differentiates them.

We next applied the method to mixtures of molecules. We concentrated on mixtures of conformational isomers that, due to their structural similarities, can be particularly challenging for conventional techniques for differentiating enantiomers (e.g. (V)CD). Their rotational spectra, however, are distinct

because the moments of inertia and thus the rotational constants are very sensitive to even small geometrical changes. As a consequence, rotational spectra offer unique molecular fingerprints that can be exploited for mixture analysis.

For simultaneous identification of the chiral signatures from both the EQ1 and EQ2 carvone conformers, MW chirps (100 and 60 MHz broad, respectively) exciting the drive transitions of both were applied sequentially to produce the MW chirp for the drive step. The timeline presented in Figure 2 shows the experimental scheme for these measurements. RF pulses resonant with the RF transitions of both

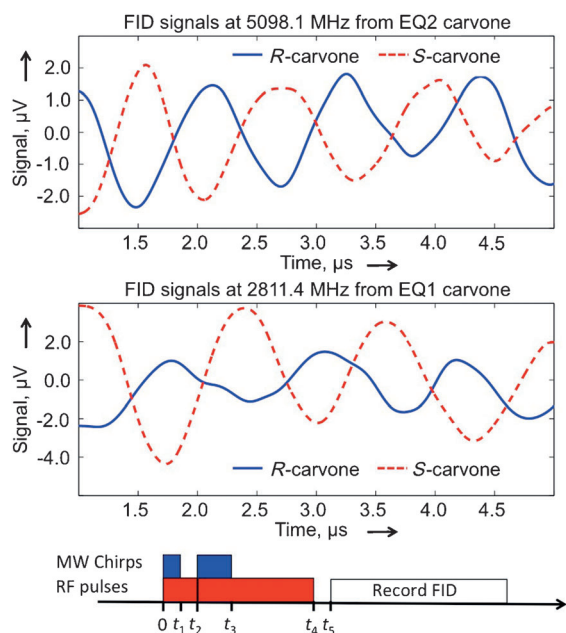


Figure 2. The downsampled free-induction decays (FIDs) of the listen transitions obtained simultaneously, clearly demonstrating the π phase difference between the enantiomers. The components of the FID signals at 5098.1 MHz (EQ2) and 2811.4 MHz (EQ1), respectively, were extracted by digital band-pass filters and are shown mixed down to about 1 MHz. Below the FIDs, a timeline shows how the excitation fields were stacked to excite both conformations simultaneously. The MW chirps were 3.0→2.9 GHz, $0.250\ \mu\text{s} = t_1$ and 5.52→5.58 GHz, $1.0\ \mu\text{s} = t_3 - t_2$. The RF pulses were 118.40 MHz, $0.500\ \mu\text{s} = t_2$ and 459.75 MHz, $5.0\ \mu\text{s} = t_4 - t_2$. The FID was recorded for $10\ \mu\text{s}$ beginning at $t_5 = t_4 + 1.2\ \mu\text{s}$.

conformers were also stacked, allowing for simultaneous, enantiomer- and conformer-selective detection. Due to the broadband capabilities (2–8.5 GHz) of our spectrometer, we could monitor the listen transitions and measure the corresponding phases for both conformations simultaneously (Table 2). Figure 2 displays the corresponding downsampled FIDs for both conformers and enantiomers of carvone. As with the single-species measurements described above, similar results were obtained under RF[−] conditions.

The absolute configuration of the molecule can be determined from the absolute phase of the listen signal and knowledge of the signs of the dipole-moment components

Table 2: Phases (in radians) of conformational mixtures of *R*- and *S*-carvone from simultaneous measurement of both EQ1 and EQ2.

	Conformer	<i>S</i>	<i>R</i>	$\Delta\phi^{[d]}$
RF ⁺	EQ1 ^[a]	−1.98	1.23	−3.21
	EQ2 ^[b]	−0.20	2.93	−3.13
RF [−]	EQ1 ^[a]	1.30	−1.87	3.17
	EQ2 ^[b]	2.96	−0.13	3.09
$\sigma^{[c]}$		0.11	0.33	0.35

[a] Drive chirp 3.0→2.9 GHz (0.250 μs), RF twist at 118.4 MHz

(0.500 μs), listen at 2811.40 MHz. [b] Drive chirp 5.52→5.58 GHz (1 μs), RF twist at 459.75 MHz (5 μs), listen at 5098.12 MHz.

[c] Uncertainty in measurement. The phases obtained from the RF[−] and RF⁺ measurements were combined for each enantiomer by subtracting or adding π radians for the *R* and *S* enantiomers, respectively. These combinations yielded a total of five measurements for *R* and four for the *S* enantiomers. [d] $\Delta\phi$ is the phase difference between enantiomers and σ is the standard deviation.

obtained from quantum chemical calculations. In our experiments on carvone, the absolute phases can be determined from the observed phases from the simultaneous measurements of both conformations. The experimentally obtained phases are shifted from the absolute phases due to the shift in the start of FID recording time (t_r) from the absolute start of the FID (t_0). This time shift is the same for both conformations with the addition of the well-defined 500 ns delay (corresponding to t_2 in the timeline of Figure 2) between excitation of the two conformations. Using this delay and the observed phases, values of t_r can be found that recover the observed phases. This procedure is described in the Supporting Information.

Although a unique value for t_r cannot be found from the current data, all of the solutions over a range of 2 μs centered at the oscilloscope trigger yield an absolute phase of $-1.62(50)$ for *S*-carvone and $+1.55(50)$ for *R*-carvone. In order to determine the absolute configuration, a prediction of the absolute phase is needed. Since the signal at the listen frequency has the form of Equation (1), where ν is the listen frequency and t is the time relative to t_0 , an absolute phase of $\pm\pi/2$ is expected.

$$\text{sig}(t) \propto |\mu_a \mu_b \mu_c| \cdot \cos\left(2\pi\nu t + \frac{\pi}{2} \left(\frac{\mu_a \mu_b \mu_c}{|\mu_a \mu_b \mu_c|} \right)\right) \quad (1)$$

The sign of the phase depends on the quantity $\mu_a \mu_b \mu_c$. From our quantum chemical calculations (B3LYP/aug-cc-pVDZ and B3LYP/aug-cc-pVTZ optimizations with Gaussian03^[15]) on both conformations, all three dipole-moment components are negative for *S*-carvone while two are negative for *R*-carvone. Therefore, an absolute phase of $-\pi/2$ is expected for *S*-carvone and $+\pi/2$ for *R*-carvone, as observed. This agreement verifies our assignment of absolute configurations.

In the final set of experiments, we measured the *ee* values of enantiomeric mixtures of carvone (Figure 3). The amplitude of the three-wave-mixing signal is proportional to the *ee*, so that this value is directly accessible with our experiments. The results are shown in Figure 3 for EQ2, comparing non-enantiopure samples with a racemic mixture and enantiomerically pure *R*-carvone. The agreement is satisfying, in

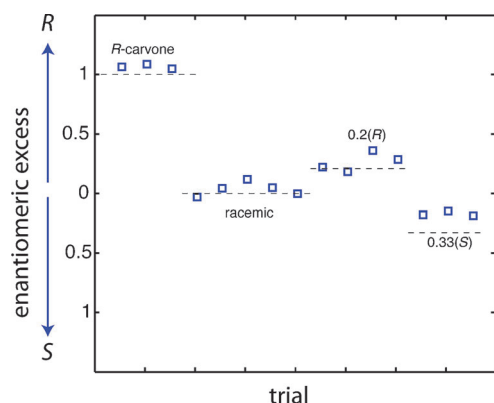


Figure 3. Repeated measurements of enantiomeric excess for carvone: enantiomerically pure *R*-carvone, the racemic mixture, and mixtures with a slight excess of *R*-carvone (0.2(*R*)) and of *S*-carvone (0.33(*S*)).

particular when one considers that the preparation of the mixture also adds uncertainty to the corresponding *ee*.

In summary, we have demonstrated molecule-sensitive enantiomer differentiation of chiral molecules by microwave three-wave mixing in a supersonic jet using broadband chirped-pulse microwave spectroscopy. Only minor changes to our existing chirped-pulse broadband rotational spectrometer were required. The extension of chiral three-wave mixing to widely used supersonic jets brings this method a significant step closer to a broader usage. We also applied this technique to analyze a mixture of conformational isomers. The intrinsically narrow lines in rotational spectroscopy and the unique, fingerprint-like spectrum of each molecule allowed us to obtain the signatures of chiral molecules even in the presence of other very similar chiral species. We also measured the *ee* values of enantiomeric mixtures. Finally, with the aid of quantum chemical calculations to obtain the sign of $\mu_a\mu_b\mu_c$, we determined the

absolute configuration of the enantiomers of carvone from the three-wave-mixing signals.

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