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Enantiomer-sensitive spectroscopy and mixture analysis of chiral molecules containing two stereogenic centers – Microwave three-wave mixing of menthone

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ABSTRACT

We demonstrate three-wave mixing for enantiomer differentiation in the microwave regime using rotational transitions in cold gas-phase samples of menthone and carvone. The technique can also be understood as a polarization-dependent double-resonance experiment and has recently been shown to have high chiral sensitivity and mixture compatibility due to its resonant character. We here apply it to molecules containing two stereogenic centers, an important step towards investigating more complex species. We also demonstrate simultaneous microwave three-wave mixing of two structurally related monoterpenes, menthone and carvone, in a chiral mixture, which is possible due to the high sensitivity of rotational spectroscopy to structural differences.

1. Introduction

Chiral molecules have fascinated chemists for more than 150 years, an early highlight being Louis Pasteur's discovery in 1849 that the phenomenon of optical rotation arose from what we now call chirality on a molecular scale [1]. This discovery is particularly notable since the three-dimensional arrangement of atoms to form molecules was not yet known. In 1874, Van't Hoff and Le Bel independently ascribed Pasteur's observation to the underlying three-dimensional geometry of chiral molecules, which can result in two non-superimposable mirror-image structures called enantiomers [2,3]. Besides their nearly identical physical properties (which makes them difficult to separate), enantiomers can have very different chemical properties and biological functions. Most drugs developed in the last decade have been of a specified chirality, and a variety of chiral analysis techniques have been developed so far [4–6]. Despite the importance of chirality in nature and our every-day life, their quantitative analysis remains challenging, in particular for more complex molecules and when mixed with other species – a very common situation in biological systems.

Partly facilitated by recent instrument developments, chiral-molecule research has become a very active field: Motivated by the need for methods that can differentiate enantiomers and unambiguously determine molecular handedness and enantiomeric excess, a number of ground-breaking new experiments have been developed and demonstrated very recently. For example, in photoelectron circular dichroism (PECD), circularly polarized laser light or synchrotron radiation are used to investigate the distribution of the ejected photoelectrons of a chiral sample [7–13]. A striking forward/backward asymmetry in the distribution of the ejected photoelectrons with respect to the propagation direction of the laser light is detected for the two enantiomers that allows for their differentiation. Very recently, Coulomb explosion imaging resulting from multiionization with high-power femtosecond laser light was used to determine the absolute configurations of the two enantiomers in a racemic mixture of CHFClBr, a promising proof-of-principle experiment [14]. The scale-up to larger molecules and, in particular, to species containing several stereogenic centers might be difficult, because fragmentation at the stereogenic center is an important prerequisite.

We recently demonstrated enantiomer differentiation, enantiomeric excess determination, and evaluation of the absolute configuration of chiral molecules in the gas phase in an international collaboration with David Patterson and John M. Doyle from Harvard University. This technique is based on broadband microwave spectroscopy and microwave three-wave mixing and can also be

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applied to chiral mixtures [15–17]. It exploits that the overall product, $\mu_a\mu_b\mu_c$, is of opposite sign between enantiomers since their dipole moments are mirrored. In the experiments, triads of transitions that include all three dipole-moment components are used. Fig. 1 shows an energy level scheme for microwave-three wave mixing using menthone as an example molecule (Fig. 2). Note that allowed rotational transitions can all be classified as purely a-type, b-type, or c-type, i.e., they solely depend on μ_a, μ_b, or μ_c. By first interrogating μ_c and μ_a with two orthogonal fields (for example oriented along the *z*- and the *y*-axes of the laboratory-fixed coordinate system given in Fig. 3), the phase of the molecular signal obtained for the listen transition involving μ_b polarized in the third, mutually orthogonal direction (along the *x*-axis, Fig. 3) differs by π radians between enantiomers due to the change in sign of $\mu_a\mu_b\mu_c$. Thus, a definitive chiral signature is obtained for non-racemic mixtures, with the signal amplitude being directly proportional to the enantiomeric excess (ee). Microwave three-wave mixing can be viewed as a polarization-sensitive double-resonance experiment and is widely applicable to polar chiral molecules with dipole-moment components $\mu_a, \mu_b, \mu_c \neq 0$ that can be brought into the gas phase.

In the present work, we apply the technique to molecules exhibiting more than one stereogenic center. The working principle of microwave three-wave mixing relies on the opposite signs of $\mu_a\mu_b\mu_c$ for the two enantiomers and also holds for molecules with multiple stereogenic centers. Complications might arise from the increasing number of stereoisomers, leading to dense and widespread spectra with low intensities. Many molecules of biological interest contain several stereogenic centers, such as the steroids cholesterol, estradiol, and testosterone, which are important as a dietary lipid and hormones. The female hormone β-estradiol, for example, has five stereogenic centers. It can be vaporized by heating, as shown in a recent report of its gas-phase UV spectrum [18] and so should also be accessible by microwave three-wave mixing. Perhaps equally importantly, as the number of stereogenic centers increases so does the molecular size, resulting also in an increased number of conformational isomers. One of the advantages of MW spectroscopy is that it is not only molecule-, but also conformer-specific.

As a prototype organic molecule with more than one stereogenic center and several low-energy conformers, we investigated the monoterpene menthone, which is an important step towards handling more complex molecules. Menthone (2-isopropyl-5-methylcyclohexanone) is naturally occurring and used in perfumery and cosmetics for its characteristic aromatic and minty odor. It is an important constituent of the essential oils of pennyroyal and peppermint. Since menthone has two asymmetric carbon atoms as stereogenic centers, $2^2 = 4$ different stereoisomers are possible (Fig. 2). They can be divided into two pairs of enantiomers: (−)- and (+)-menthone ((*2S,5R*)-2-isopropyl-5-methylcyclohexanone and (*2R,5S*)-2-isopropyl-5-methylcyclohexanone) as well as (−)- and (+)-isomenthone ((*2S,5S*)-2-isopropyl-5-methylcyclohexanone and (*2R,5R*)-2-isopropyl-5-methylcyclohexanone). The respective menthone and isomenthone structures are diastereomers with respect to each other. For menthone, the methyl and the isopropyl groups are given in *trans* arrangement with respect to each other, while for isomenthone they exhibit a *cis* arrangement. In nature, (−)-menthone is most abundant.

Generally, diastereomers differ in their physical and chemical properties, so that they can be easily distinguished by, for example, their melting and boiling points and their rotational constants. Rotational spectra are molecular fingerprints due to their high sensitivity to structural changes and inherently high resolution, such that even very structurally similar species, such as conformers, can be unambiguously distinguished and assigned. We use these features to our advantage in microwave three-wave mixing. In Fig. 2, we give the rotational constants for two diastereomeric pairs

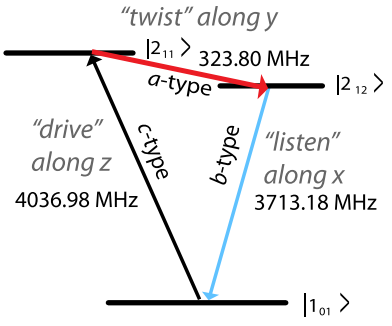

Fig. 1. Energy level scheme for microwave three-wave mixing of menthone: A triad of rotational energy levels connected by a-, b-, and c-type transitions is involved in the excitation (drive and twist) and the detection scheme (listen transition). The polarization directions of the respective excitation fields and the detection are also given and correspond to the laboratory-fixed coordinate system shown in Fig. 3.

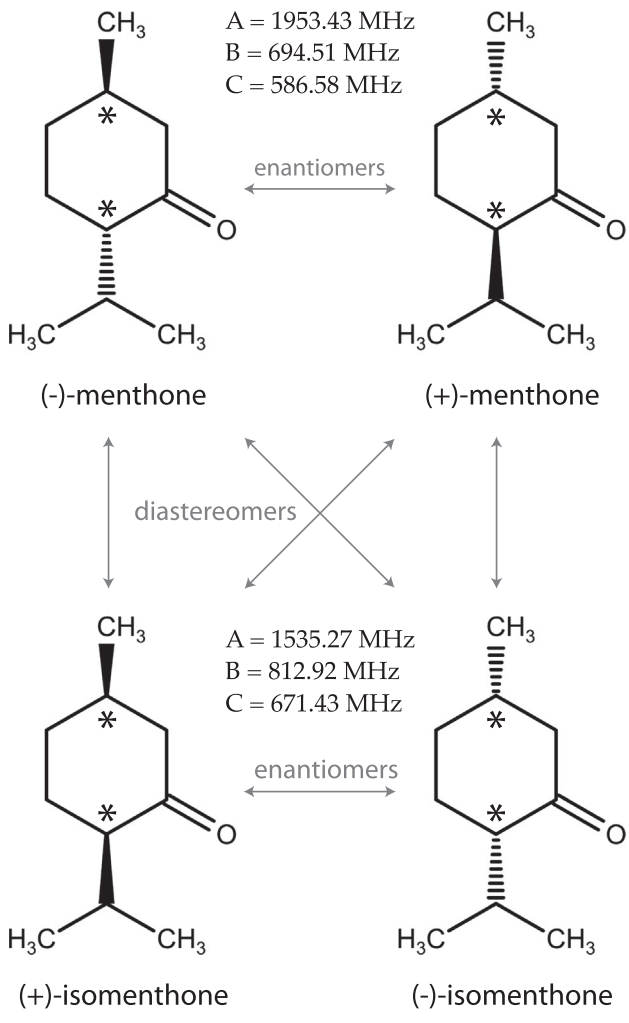

Fig. 2. Schematic molecular structures of the four stereoisomers of menthone (2-isopropyl-5-methylcyclohexanone): (−)-menthone and (+)-menthone as well as (−)-isomenthone and (+)-isomenthone are enantiomers of each other. The stereogenic centers are marked with asterisks. Menthone and isomenthone structures are diastereomers, respectively, which can be differentiated by their rotational spectra. Rotational constants for one conformer of both diastereomers obtained from a rough fit to our broadband rotational spectra are also given for both diastereomers to illustrate their clear difference.