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Stereochemical analysis of (+)-limonene using theoretical and experimental NMR and chiroptical data

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Using limonene as test molecule, the success and the limitations of three chiroptical methods (optical rotatory dispersion (ORD), electronic and vibrational circular dichroism, ECD and VCD) could be demonstrated. At quite low levels of theory (mpw1pw91/cc-pvdz, IEFPCM (integral equation formalism polarizable continuum model)) the experimental ORD values differ by less than 10 units from the calculated values. The modelling in the condensed phase still represents a challenge so that experimental NMR data were used to test for aggregation and solvent–solute interactions. After establishing a reasonable structural model, only the ECD spectra prediction showed a decisive dependence on the basis set: only augmented (in the case of Dunning's basis sets) or diffuse (in the case of Pople's basis sets) basis sets predicted the position and shape of the ECD bands correctly. Based on these result we propose a procedure to assign the absolute configuration (AC) of an unknown compound using the comparison between experimental and calculated chiroptical data.

1. Introduction

In the field of configurational analysis it is very important to use structural information since only then a reliable assignment of the absolute configuration of new molecules is possible [1,2]. Despite enormous progress in instrumentation and methods [3] structural analysis is still a challenge, illustrated by the review of Nicolaou and Snyder [4] who presented data based on which roughly 1000 articles published between 1990 and 2004 have to be revised because of structural reasons. Likewise, Maier [5] presented numerous recent examples of erroneous chemical formulas. One of the early configurational assignment strategies is based on the chiral recognition of unsaturated compounds with optically active lanthanide complexes measured by NMR. Offermann and Mannschreck [6] used limonene to demonstrate that the changes in chemical shifts by titrating the analyte with chiral lanthanide complexes can be used to determine the enantiomeric excess.

The chiral monoterpene limonene is an important starting material in chemical synthesis [7–9]. Moreover, it is used in the fragrance and food industry, and is an important synthetic material [10]. Limonene is biotransformed by a variety of organisms such as bacteria, fungi, and plants [11]. In this way, using appropriate species a number of synthetically important metabolites can be produced. One of the biosynthetic enzymes, limonene epoxide hydrolase, was recently used as test case for asymmetric biocatalysis [12].

Limonene has served as chiral test molecule to investigate chiroptical methods such as vibrational circular dichroism (VCD), electronic circular dichroism (ECD), and optical rotatory dispersion (ORD), and even as inducer of chirality. After the early report of Noack [13] using the monoterpene menthol, Kobayashi et al. [14], Fukiji et al. [15a] and Aimi et al. [15b] were able to induce optical activity in achiral environments by limonene enantiomers. A review summarizes solvent chirality transfer in the field of supramolecular chemistry [15c]. In recent publications, the orientation of limonene enantiomers was measured by sum frequency vibrational spectroscopy, detecting surface chirality [16].

In our report, we present a detailed analysis of spectroscopic data about (+)-limonene in order to (i) deliver detailed analytical data about the academically and industrially important monoterpene limonene and (ii) improve the methodology of chiral analysis. Based on the results for strychnine base and the protonated/HCl form [17,18], we further explore the success and limitations of the absolute configuration (AC) determination using a comparison of experimental and calculated chiroptical data. Two recent publications nicely illustrate the importance of solvent

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modelling for the interpretation of chiroptical data [19a]. Consequently, we will discuss the influence of solvent models on the determination of the absolute configuration.

NMR and chiroptical data about limonene are combined, and all three major chiroptical methods (VCD, ECD, ORD) are applied. The importance to use more than one chiroptical method to determine the absolute configuration of a compound has been convincingly put forward by Polavarapu [19b]. As an example, the AC of two nonylphenols was determined by optical rotation and VCD analysis [20]. For one of the compounds (NP112) it is still unclear if the AC was correctly assigned due to impurity problems. The AC assignment of nonylphenol NP35 [20] was confirmed by x-ray analysis [21].

The present report is organized in the following way: First, the structural models of the relevant conformers are obtained together with their populations; second, population-weighted chiroptical data are calculated for the structural models and compared to experimental values; third, conclusions derived from these comparisons are critically assessed.

2. Results

In order to develop a general procedure to analyse the configuration of compounds for which NMR data are available, limonene is a good choice since it serves as test molecule for NMR method development. For chemical shifts we used information from Skakovskii et al. [22] and Bohlmann et al. [23] In addition, deuterium NMR helped in assigning diastereotopic protons [24]. Assuming similar trends for both nuclei, protons and deuteriums, the calculations unambiguously assigned the methylene protons of limonene.

2.1. Structural models

Limonene represents a simple monoterpene (Fig. 1). At first sight, it might be conformationally flexible in the six-membered ring. The conformational search (Discovery Studio, Accelrys [25], Universal forcefield) of a model for *R*-limonene delivered two types of ring conformers which differed in the orientation of the isopropenyl substituent: equatorial or axial. However, the axial orientation of the isopropenyl substituent raises the computed energy substantially. Since the energy difference to the conformers with an equatorial isopropenyl substituent was larger than 2.5 kcal/mol, we can safely assume that almost exclusively the latter form exists in solution. Therefore, conformational averaging due to ring inversions cannot be taken as explanations in the course of spectrum interpretation [24]. It is more likely, that the rotation of the isopropenyl group is responsible for averaged signals in NMR spectra.

Having established the correct form with the isopropenyl group in the equatorial position, a full systematic conformational search was conducted using Discovery Studio. With the applied universial forcefield ten possible conformers were obtained with a cut-off of 2.5 kcal/mol higher than the lowest energy conformer. All ten were further geometry optimized with Gaussian09 [26] (mpw1pw91/cc-pvdz; IEF-PCM: chloroform). During this step, only three optimized conformers remained (Fig. 2A–D) indicating that most often nonstable conformations were generated in the forcefield search. Frequency calculations confirmed that all three are minimum structures due to the absence of vibrations with imaginary frequencies. The ring dihedrals only differ slightly among the isopropenyl rotamers, with differences smaller than 5° (Table 1). The isopropenyl dihedrals are similar (differences smaller than 4°) to the dominant conformers calculated by Debie: [27] +132, −142, and −8 for conformer 1,2 and 3, respectively, which were obtained on the B3LYP/6-31g(d) level of theory in vacuo. For comparison, the axial conformer at lowest energy is also shown in Table 1. It is 1.9 kcal/mol higher in free energy compared to the highest in energy equatorial conformer (conformer 1) calculated at the mpw1pw91/cc-pvdz; IEF-PCM: chloroform level of theory.

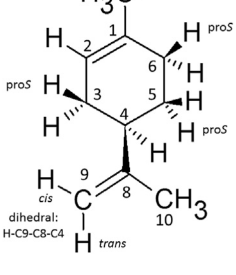

Fig. 1. (+)-*R*-limonene.

2.2. Population analysis

We followed two approaches for population analysis starting with the purely computational one. The three conformers differed in free energies depending on which level of theory and which solvent was used for modelling. Different levels of theory and two solvents (ethanol and chloroform) were used for frequency calculations and the free energy values are presented in Table 2. Using the Boltzmann equation we calculated the populations for the three conformers (Table 3). Since data with neat limonene have to be interpreted, it is important not to use only one solvent that might be an inappropriate substitute for limonene as solvent. So two largely different solvents and two different basis sets were used and the arithmetic mean for the calculated populations based on free energies were determined for conformer 1, 2, and 3 as 28%, 38%, and 34%, respectively. The standard deviations of the calculated populations are 3%, 6%, and 3%, respectively, which fits nicely to results from literature e.g. glucose [28]; *endo*-borneol [29]; cylohexene oxides [30]. Interestingly, the calculated entropies are highest for conformer 2 at all levels of theory (Table 2).

Populations derived from calculated enthalpies differ (Tables 2, and 3) in that conformer 2 is less populated due to the neglect of entropic contributions (34:23:43). Since calculated entropies might be unreliable due to a large error associated with vibrations at low wavenumbers (<200 cm^{-1}) it is not easy to decide if free energies or enthalpies deliver accurate energies for the population analysis. Applying a reasonable scaling factor in the thermochemical analysis does not solve the problem. Based on the literature [31–33] we can assume, that for the levels of theory in this study (mpw1pw91 as a functional, and the Dunning's basis sets) an average scaling factor of 0.97 can be assumed. Please note that Irikura et al. [34] suggested that only two digits are meaningful. As an example, using a scaling factor to zero-point vibrational energies and thermal corrections to enthalpies of 0.97, gives for the mpw1pw91/aug-cc-pvtz/IEFPCM (chloroform) level of theory the following populations for conformer 1, 2, and 3: 31%, 28%, and 41%, which differ to the values obtained using unscaled free energies or enthalpies.

The second approach for the population analysis represents a combination of experimental values and calculated data. Based on the three structural models for the conformers, we set out to calculate ^{13}C chemical shifts. Experimental data are readily available from literature which is methodologically important since we want to draw conclusions from this study for the analysis of other molecules for which published values are available. In case of unstable molecules, or natural products for which no natural sources