Absolute Configuration

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Is Enantiomer Assignment Possible by NMR Spectroscopy Using Residual Dipolar Couplings from Chiral Nonracemic Alignment Media?—A Critical Assessment**

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The discovery of Jean-Baptiste Biot in 1815 that optical activity is not a property bound to a certain aggregation state of matter but a property of the constituting molecules themselves, has had an enormous influence on the structural models that chemists developed at the end of the 19th century, long before the description of the chemical bond was based on quantum mechanics. Pasteur achieved the first separation of enantiomers in 1847,[1] namely by crystallization of a racemic tartrate mixture that separated the two enantiomers into enantiomorphic crystals, solutions of which rotated the plane of linearly polarized light in opposite directions. Not until 1951, when Bijvoet used anomalous X-ray diffraction,[2] it was possible to assign the absolute configuration to a specific enantiomer. However, anomalous X-ray diffraction has not put the problem of assigning absolute configurations to rest, because many chemical compounds cannot be crystallized. Moreover, anomalous X-ray diffraction of molecules that consist exclusively of lightweight atoms commonly lacks the needed accuracy to allow unambiguous assignment of absolute configurations.[3] An alternative method for resolving enantiomers is to convert them to diastereoisomers, either by chemical derivatization with chiral nonracemic moieties or by intermolecular coordination with chiral nonracemic reagents. In this way it is possible to determine absolute configuration from NMR observables, most commonly chemical shifts.[4] The use of chiroptical spectroscopies such as optical rotatory dispersion, and electronic or vibrational circular dichroism is well established, sometimes in combination with ab initio calculations.[5] Further methods are conceivable but impractical momentarily.[6] Yet, there is currently not a simple and universally applicable approach to determine the absolute configuration of molecules with few stereogenic centers.

Two recent papers published in 2007 and 2011 have therefore created a lot of excitement in the chemistry and NMR spectroscopy communities. Their titles are: „Stereochemical Identification of (*R*)- and (*S*)-Ibuprofen Using Residual Dipolar Couplings, NMR, and Modeling“,[7] henceforth called „article 1“, and more recently: „Spin-Selective Correlation Experiment for Measurement of Long-Range *J* Couplings and for Assignment of (*R*/*S*) Enantiomers from the Residual Dipolar Couplings and DFT“,[8] henceforth called „article 2“.

Both articles describe the assignment of the absolute configuration of the chiral molecules, ibuprofen **1** (article 1) and 4-methyl-1,3-dioxolan-2-one **2** (article 2), using NMR spectroscopy in chiral nonracemic alignment media (Figure 1). Under chiral nonracemic conditions, the authors measured residual dipolar couplings (RDCs), a NMR parameter only visible in oriented samples, such as in liquid crystals, but not in isotropic solvents.

The interaction of the enantiomers with the chiral nonracemic alignment medium gives rise to diastereomorphic associates for which reason the authors indeed found different sets of anisotropic parameters for each enantiomer, in total agreement with what has been previously described by some of us; for example, on quadrupolar splittings of α-pinene.[9]

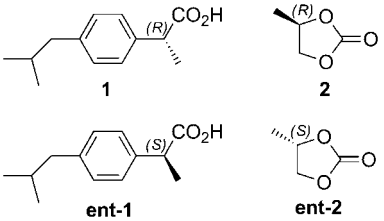

Figure 1. Molecules for which the determination of the absolute configuration by NMR spectroscopy in chiral nonracemic alignment media has been reported: ibuprofen **1** and 4-methyl-1,3-dioxolan-2-one **2**.

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From these two different sets of anisotropic parameters for the two enantiomers, the authors of articles 1 and 2, reported that they were able to assign their respective absolute configuration.

In this critique we would like to clarify some points and demonstrate that some of the procedures used in these articles are either technically unconvincing (article 1) or contradict principles of physics (article 2). To guide the reader through the critique, let us first give a brief summary: We discuss the alignment process in chiral nonracemic alignment media for enantiomers which leads to different anisotropic parameters both for rigid and flexible molecules. We then point out that an assignment of the absolute configuration of enantiomers from these NMR data could be possible if either of the two methods were successfully applied: 1) Either the alignment tensor of the enantiomers can be predicted from first principles for flexible or rigid molecules or 2) the different ensembles of conformations for enantiomers due to the alignment process can be predicted from first principles. We come to the conclusion that neither of the two methods has been demonstrated beyond doubt in the two criticized publications.

Enantiomers interact differently but still weakly with chiral nonracemic alignment media such as organic solutions of poly-γ-benzyl-L-glutamate (PBLG) or others with similar properties (diastereomorphous objects are generated as a cause of this interaction), therefore producing different sets of anisotropic magnetic interactions such as RDCs (D_{ij}) or quadrupolar couplings ($\Delta\nu_Q$).[9–10] We assign $D_{Rij}^{\exp}$ (or $\Delta\nu_{QS}^{\exp}$, in case of quadrupolar couplings) to the experimental dataset for the R enantiomer, and $D_{Sij}^{\exp}$ ($\Delta\nu_{QS}^{\exp}$) to the one corresponding to the S enantiomer. Let us consider first, for simplicity, a rigid[11] molecule like α-pinene (Figure 2),[9] where the coordinate vectors for each internuclear vector $\vec{r}_{ij}$ in the R and S enantiomers can be related by inversion and therefore Equation (1) is valid.

$$\vec{r}_{Rij} = -\vec{r}_{Sij} \tag{1}$$

This equation holds of course not only for enantiomers of rigid molecules but in general for enantiomorphous conformations.[12]

The RDC (D_{ij}) value between nuclei i and j is given, in tensorial form,[13] by Equation (2),

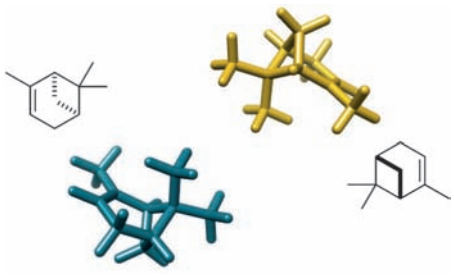

Figure 2. Structures of the enantiomers of α-pinene. The 3D coordinates of the two enantiomers of rigid molecules are inversion symmetric; that is every atom coordinate (x, y, z) is replaced by coordinate ($-x$, $-y$, $-z$). This is true irrespective of the chiral nonracemic alignment medium. For flexible molecules, enantiomers may adopt non-enantiomorphous conformations.

$$D_{ij} = -\frac{3\gamma_i\gamma_j\mu_0\hbar}{8\pi^2 r_{ij}^3}\vec{r}_{ij}^{\,T}\hat{A}\vec{r}_{ij} \tag{2}$$

where $\vec{r}_{ij}$ is a unit vector connecting nuclei i and j, γ_i and γ_j are the corresponding magnetogyric ratios, and μ_0 and $\hbar$ are physical constants. From Equation (2) it is obvious that replacement of $\vec{r}_{ij}$ by $-\vec{r}_{ij}$ does not change the value of D_{ij}.

The alignment tensor $\hat{A}$, identical to 2/3 of the Saupe matrix $\hat{S}$,[13] is a 3×3 traceless symmetric matrix, which contains the information about the rotational distribution of the vector $\vec{r}_{ij}$, that is, encodes the probability of the vector pointing in a particular direction of space. For a rigid molecule such as α-pinene this distribution is the same for all internuclear vectors (excluding trivial methyl internal rotations) and therefore the tensor can be associated to the whole molecule.

$\hat{A}$ can be determined by minimizing (for example in a least square sense) the difference between the observed $D_{ij}^{\exp}$ and the back-calculated D_{ij}^{fit} as a function of the matrix elements of $\hat{A}$. This difference is commonly expressed as the Cornilescu[14] quality factor Q defined in Equation (3).

$$Q = \sqrt{\frac{\sum_{ij}\left(D_{ij}^{\exp} - D_{ij}^{\mathrm{fit}}\right)}{\sum_{ij}\left(D_{ij}^{\exp}\right)^2}} \tag{3}$$

Q is ideally zero, when the back-calculated D_{ij}^{fit} are identical to the experimentally measured RDCs $\left(D_{ij}^{\exp}\right)$. Since there is some noise in the measured RDCs $\left(D_{ij}^{\exp}\right)$ as well as in the structural model because of geometric inaccuracies in the method employed for molecular modeling, and vibrational corrections may not be taken into account, the quality factor Q is normally larger than zero.

That rigid enantiomers provide different sets of RDCs $\left(D_{Rij}^{\exp} \neq D_{Sij}^{\exp}\right)$ in chiral nonracemic alignment media is due to the fact that the alignment tensor experienced by the R enantiomer $\hat{A}_R$ is different from the one experienced by the S enantiomer $\hat{A}_S$. The expected RDCs for the R enantiomer $\left(D_{Rij}^{theo}\right)$ and for the S enantiomer $\left(D_{Sij}^{theo}\right)$ are therefore different [Eq. (4)]:

$$\begin{aligned} D_{Rij}^{theo} &= -\frac{3\gamma_i\gamma_j\mu_0\hbar}{8\pi^2 r_{ij}^3}\vec{r}_{ij}^{\,T}\hat{A}_R\vec{r}_{ij} \\ &\neq -\frac{3\gamma_i\gamma_j\mu_0\hbar}{8\pi^2 r_{ij}^3}\left(-\vec{r}_{ij}^{\,T}\right)\hat{A}_S\left(-\vec{r}_{ij}\right) = D_{Sij}^{\mathrm{theo}} \end{aligned} \tag{4}$$

Revisiting the two enantiomorphous 3D structures of a rigid molecule, such as the enantiomers of α-pinene, we assume that we have measured two sets of RDCs: $D_{Rij}^{\exp}$ for the R enantiomer, and $D_{Sij}^{\exp}$ for the S enantiomer; and these two sets contain different RDCs. Let us focus first on the RDC dataset and investigate the result of fitting the R or S configuration of the rigid molecule in an attempt to discriminate between the two of them. We call the resulting Q values: Q_{RR} for fitting the R enantiomer to the experimental RDCs observed for the R enantiomer and Q_{RS} [Eq. (5)] for fitting the S enantiomer to the same experimental RDCs.

$$Q_{RS} = \sqrt{\frac{\sum_{ij} \left(D_{Rij}^{\mathrm{exp}} - D_{Sij}^{\mathrm{fit}}\right)^2}{\sum_{ij} \left(D_{Rij}^{\mathrm{exp}}\right)^2}} \quad (5)$$

As mentioned above, the fitted RDCs are the same for a molecule described by $\vec{r}_{ij}$ as for a molecule described by $-\vec{r}_{ij}$. Since for a rigid molecule all vectors in the two enantiomers fulfill $\vec{r}_{Rij} = -\vec{r}_{Sij}$, Q_{RR} and Q_{RS} must have identical values; and by the same argument Q_{SR} and Q_{SS} are also identical. Hence, absolute assignment of enantiomers (from RDCs) is not possible when they have enantiomorphous 3D structures.

However, for chiral, flexible molecules such as ibuprofen **1** or 4-methyl-1,3-dioxolan-2-one **2**, it is conceivable that the interaction of the chiral nonracemic alignment medium with each enantiomer could lead to differences in the conformational space distributions for each of them leading to different populations for enantiomorphous conformations. However, in article 2, the authors calculate with DFT the conformations of the two enantiomers without taking into account the possible effects of the chiral nonracemic alignment medium.[15] Thus, they should necessarily find conformations for the enantiomers that are mirror images of each other $(\vec{r}_{Rij} = -\vec{r}_{Sij})$, leading to identical fits (Q-values) for the RDCs and making impossible the assignment of the absolute configuration to each enantiomer. That the Q-values are different, as described in article 2 (page 6872), suggests that there is either a flaw in the DFT calculations, resulting in diastereomorphous conformations for the enantiomers, or a possible problem in the fitting procedure.

In the case of flexible molecules, the conformational space adopted by each enantiomer during their diastereomorphous interactions with the chiral nonracemic alignment medium could be different in principle. By this argument, the unequivocal assignment of absolute configuration may be possible, provided that these interactions could be predicted accurately. If the alignment is dominated by non-enantiomorphous conformations and they are correctly calculated, one would find Equation (6):

$$\begin{aligned} &Q_{RR} < Q_{RS} \\ &\text{and} \\ &Q_{SS} < Q_{RS} \end{aligned} \quad (6)$$

This is the procedure described in the Results section of article 1: The conformational ensemble was calculated taking the alignment medium into account by including the electrostatic interactions between PBLG and ibuprofen. Then two non-enantiomorphous conformations were derived, which both fitted well to the experimental RDCs fulfilling the inequalities given in Equation (6). While in principle this approach might work, there are several critical issues with article 1: a) the procedure eliciting how to obtain the different conformations for the enantiomers is not described (force fields used, structure of PBLG used, protocol), b) the coordinates of the non-enantiomorphous conformations are not deposited, such that the fits (Q-values) of the dipolar couplings cannot be reproduced, which would be important for checking the inequalities in Equation (6), c) the entire conformational space of ibuprofen has not been explored (only two out of a total of four flexible bonds were varied), and d) enantiomer differentiating interactions with chiral media are not uncommon in, for example, chromatography, however, properties depending on these interactions such as the order of elution in chiral stationary phases, are difficult to calculate for flexible molecules.[16] Thus it remains highly doubtful whether the approach described in article 1 is feasible. In particular, by not describing how the interaction between the chiral molecule and the chiral environment is treated, the key of the problem is not addressed.

We conclude that it is a highly attractive goal to assign enantiomers using anisotropic NMR parameters acquired in chiral nonracemic alignment media. This is possible in principle, because these parameters vastly differ for enantiomers either flexible or rigid. If a theory is advanced enough to faithfully predict the miniscule change in a conformation or its population that may be induced by a chiral alignment medium, then the quality of the fits of the experimental RDCs to those back-calculated from the predicted conformations will yield the enantiomer assignment. This approach requires conformationally flexible molecules. An approach specifically geared towards flexible molecules would be beneficial since they are less easily addressed with other techniques. For rigid, chiral molecules, for which the 3D structure of two enantiomers are enantiomorphous, a different method must be used and could be used also for flexible molecules. This procedure could be as follows: Predict—potentially with the help of atomistic simulations—the different alignment tensors $\hat{A}_R^{\mathrm{theo}}$ and $\hat{A}_S^{\mathrm{theo}}$ of enantiomers under chiral nonracemic alignment conditions and then compare with Equation (7):

$$D_{Rij}^{\mathrm{theo}} = \vec{r}_{ij}^{\,T} \hat{A}_R^{\mathrm{theo}} \vec{r}_{ij} \text{ and } D_{Sij}^{\mathrm{theo}} = \vec{r}_{ij}^{\,T} \hat{A}_S^{\mathrm{theo}} \vec{r}_{ij} \quad (7)$$

Prediction of $\hat{A}_R^{\mathrm{theo}}$ and $\hat{A}_S^{\mathrm{theo}}$ of enantiomers under chiral nonracemic alignment conditions has not been achieved thus far and assignment of the absolute configuration by NMR spectroscopy is still an open problem. Which of the two procedures or another ingenious method not discovered yet will be successful remains to be seen in the future.

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