Configuration Determination

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Residual Chemical Shift Anisotropy (RCSA): A Tool for the Analysis of the Configuration of Small Molecules**

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During the last decade anisotropic nuclear magnetic resonance (NMR) parameters measured in weakly aligned samples have had a profound impact on the structure determination of bio-macromolecules[1] and small-to-medium-sized organic molecules.[2] Almost exclusively, residual dipolar couplings (RDCs) have been used for the analysis of the conformation,[3] configuration,[4] and constitution,[5] while residual chemical shift anisotropies (RCSAs) as additional parameters have only been used with proteins and nucleic acids.[6] The development of the field has mainly been determined by the availability of suitable, very weakly aligning media. One of the most important classes of alignment media is based on strain-induced alignment in a gel (SAG), for which polymer gels for water[7] and organic solvents[4b,8] have been adapted. Especially in combination with a rubber-based stretching device developed by Kuchel et al.[9] and further improvements by Kummerlöwe et al.,[10a,b] or by the method developed by Gil et al.,[10c] polymer gels allow tunable and reversible alignment strengths with conventional high-resolution NMR spectroscopy equipment.

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While RDCs provide information on the orientation of internuclear vectors, RCSAs report on the orientation of the chemical shielding tensor of individual atoms in the molecule. In contrast to RDCs, RCSAs have not yet been used for the determination of the configuration of small molecules. This is due to the fact that RDCs can be easily extracted as the difference between the total observed couplings $T = J + D$ in aligned and non-aligned conditions using the very weak dependence of ^1J couplings on the sample conditions. RCSAs, however, that are obtained as the difference in chemical shift between the aligned and non-aligned solution suffer from additional effects on the isotropic chemical shift, because the sample changes from isotropic (sample without alignment medium) to anisotropic conditions (sample with alignment medium).[11] These alignment-media-induced uncontrollable variations in the isotropic chemical shifts have to date prevented the accurate extraction of the RCSAs and could only recently be resolved by the use of variable-angle sample spinning (VASS)[12] and variable-angle (VA) NMR spectroscopy.[13] However, variable-angle spinning requires specific equipment that is not available in many laboratories. Therefore, alternative approaches are in need.

Herein, we describe an approach to reliably measure differences of RCSAs between nuclei of interest (k) and a reference nucleus (*ref*) in the same alignment medium under two aligning conditions 1 and 2. We call these double differences ΔΔRCSA. We use the rubber-based stretching apparatus to obtain the two aligning conditions 1 and 2 without changing the alignment medium and interpret the ΔΔRCSAs structurally using CSA tensors calculated from density functional theory (DFT) combined with the gauge-independent atomic orbital (GIAO) methodology for nuclei k and *ref*.[14] Furthermore, we incorporated ΔRDCs determined as the difference between the two aligning conditions in the analysis.

As alignment medium we employed the DMSO-compatible (*S*)-2-acrylamido-1-propanesulfonic acid gel (APS).[15] The two different degrees of alignment, represented by the alignment tensors $A_{ij}^{(1)}$ and $A_{ij}^{(2)}$, were achieved by two different degrees of stretching of the gel inside the stretching device. This approach ensures that the sample composition changes only minimally, if at all, between the different alignment conditions, and the observed chemical shift changes are therefore caused mainly by the RCSAs and by changes in the overall magnetic susceptibility of the sample, which affects all resonances in the same way. The latter are removed by the referencing procedure described below (for a general discussion of several other referencing methods see e.g. reference [13]). The ΔΔRCSAs (ΔRDCs of CH groups)

are measured by subtracting the chemical shifts $\delta^{(1,k)}-\delta^{(1,ref)}$ and $\delta^{(2,k)}-\delta^{(2,ref)}$ (the CH doublet splitting $T_{kl}^{(1)}$ and $T_{kl}^{(2)}$) measured under the two stretching conditions, respectively, while referencing to one resonance of the molecule. k and l are two nuclear spins, and γ_k ($r^{(k)}$) and γ_l ($r^{(l)}$) are their gyromagnetic ratios (coordinate vectors). The resulting anisotropic parameters can be written as in Equations (1) and (2)

$$\Delta\Delta\mathrm{RCSA_k(ppm)} = \left(\delta^{(1,k)} - \delta^{(1,\mathrm{ref})}\right) - \left(\delta^{(2,k)} - \delta^{(2,\mathrm{ref})}\right) = \sum_{i=x,y,z}\sum_{j=x,y,z}\left(A_{ji}^{(1)} - A_{ji}^{(2)}\right)\left(\delta_{ji}^{\mathrm{CSA}_k} - \delta_{ji}^{CSA_{\mathrm{ref}}}\right) \quad (1)$$

$$\Delta\mathrm{RDC_{kl}} = T_{kl}^{(1)} - T_{kl}^{(2)} = -\frac{3\hbar\mu_0\gamma_k\gamma_l}{8\pi^2\left(r^{(k)} - r^{(l)}\right)^5}\sum_{i=x,y,z}\sum_{j=x,y,z}\left(A_{ij}^{(1)} - A_{ij}^{(2)}\right)\left(r_j^{(\mathrm{k})} - r_j^{(\mathrm{l})}\right)\left(r_i^{(\mathrm{k})} - r_i^{(\mathrm{l})}\right) \quad (2)$$

To demonstrate the potential of our approach, we selected a configuration determination problem where the answer is already known: natural estrone and its epimer 13-*epi*-estrone (Figure 1). Since only the D ring is attached differently (*trans* for estrone, *cis* for 13-*epi*-estrone), very few ΔRDCs and ΔΔRCSAs will change significantly for the two epimers. The experimental ΔΔRCSAs and ΔRDCs were obtained only for estrone, and we compare them with back-calculated values from both structures. We show that the ΔΔRCSAs and ΔRDCs together differentiate estrone from its C13 epimer, but neither of the two parameters individually could reliably distinguish between the two epimers based on a comparison of the Q factors.

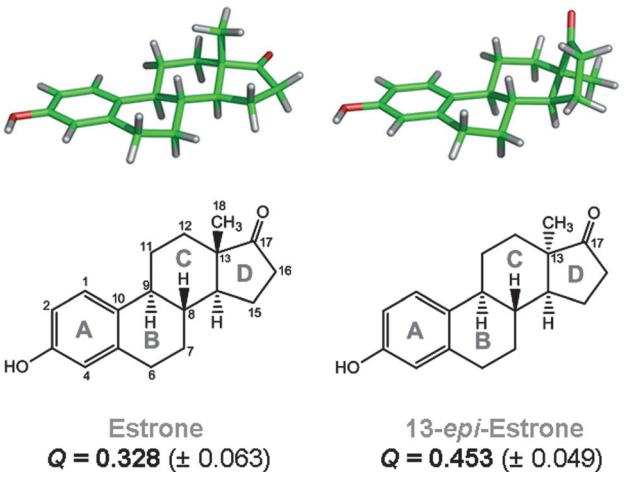

Figure 1. Structures of estrone (left) and 13-*epi*-estrone (right), respectively, and their corresponding Q values based on ΔΔRCSAs and ΔRDCs. The Q value is significantly smaller for the correct epimer (estrone) than for 13-*epi*-estrone. The standard deviation (in brackets) is explained in the text.

Proton-decoupled ^{13}C NMR spectra were recorded from a sample of estrone dissolved in [D_6]DMSO and in the presence of the APS gel (see the Supporting Information and Figure S1). Selected peaks from the spectra before and after stretching with the stretching apparatus are shown in Figure 2. We take C8 as the reference nucleus for [Eq. (1)]. In principle, it is not important which nucleus is chosen for reference; however, in practice it makes sense to select the nucleus that has the smallest CSA tensor and is therefore expected to have one of the smallest ΔΔRCSAs. As expected, the biggest ΔΔRCSA values were observed for carbon atoms belonging to aromatic and carbonyl groups. For instance, the ΔΔRCSA of the carbonyl carbon atom (C17) amounts to +5.9 Hz and that of C3 to +8.9 Hz at a ^{13}C spectrometer frequency of 150 MHz. Altogether, 13 ΔΔRCSAs were obtained, but only six ΔRDCs could be extracted reliably owing to strong coupling artifacts (see the Supporting Information).

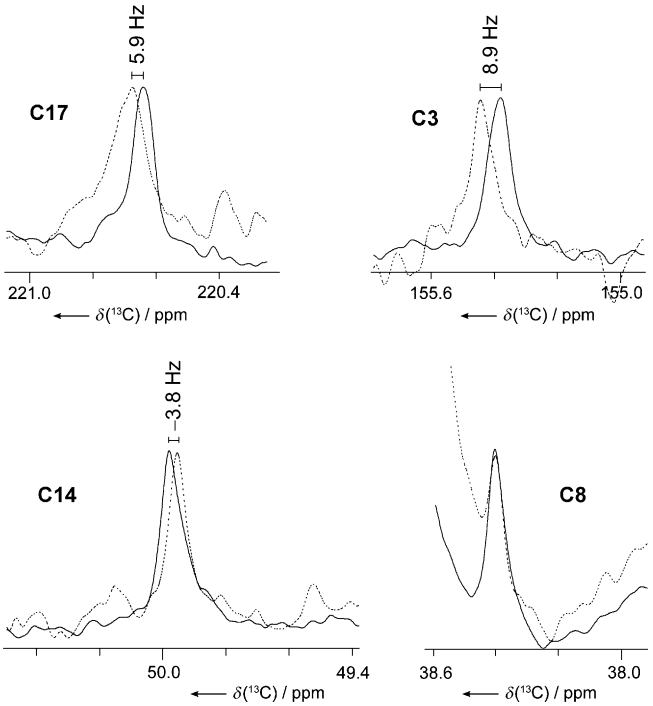

Figure 2. Excerpts from the ^{13}C-{^{1}H} 150 MHz NMR spectra from estrone before (——) and after stretching (•••••). Size and sign of ΔΔRCSAs are indicated. C8 was taken as reference.

The chemical shift tensors required for Equation (1) were computed using DFT at the GIAO//B3LYP/6-311+G(d) level on estrone and 13-*epi*-estrone B3LYP/6-311+G(d) geometries as shown in the Supporting Information.[16] The alignment tensor increment ($A_{ij}^{(1)}-A_{ij}^{(2)}$) was obtained by least-squares singular value decomposition[17] using the experimental 13 ΔΔRCSAs and six ΔRDCs and the computed chemical shift tensors according to Equations (1) and (2) (see the Supporting Information for technical details). The quality of the fit was calculated by back computation of ΔRDCs and ΔΔRCSAs using Q values.[6a] These computations were performed using a modified version of the MSpin program[18] where RDCs from methyl groups were averaged using a reported procedure.[19] The experimental error was taken into account using a Monte Carlo bootstrapping procedure (see the Supporting Information for details).

Figure S3 in the Supporting Information shows the correlations between the back-calculated ΔΔRCSAs and ΔRDCs and the experimental ones for the two configurations. The two epimers can reliably be discriminated based on the Q factors (Figure 1). The Q factor is considerably smaller for the correct epimer ($Q = 0.328 \pm 0.063$ for estrone) than for the wrong one ($Q = 0.453 \pm 0.049$ for 13-*epi*-estrone). This difference is significant compared to the Q-value standard deviation induced from the statistical errors of the ΔΔRCSAs and the ΔRDCs (see the Supporting Information). Furthermore, the fact that only small structural differences exist between the two epimers, namely at the C/D ring junction and at the D ring (see Figure 1) renders this difference even more remarkable. A difference of more than 0.1 between two Q factors was successfully used to discriminate diastereomers in earlier cases such as sagittamide.[4b]

From the six experimentally obtained ΔRDCs, only four corresponded to non-parallel C–H vectors, and therefore ΔRDC values alone cannot be used to distinguish estrone and 13-*epi*-estrone. With ΔΔRCSAs as the only input parameters, indistinguishable Q values of 0.352 ± 0.056 and 0.339 ± 0.057 are obtained for estrone and 13-*epi*-estrone, respectively. The incapability of ΔΔRCSAs to discriminate between the two diastereomers must be attributed to the linear dependence of the chemical shift tensors as large condition numbers (ca. 20) are obtained in the singular value decomposition (SVD) when only ΔΔRCSAs are used. The condition number drops to about 6 when ΔRDCs are also included. This finding clearly shows that the joint measurement and interpretation of ΔΔRCSAs and ΔRDCs improves nonlinearly the reliability of the configurational assignment. The condition number is a measure for the linear independence of the anisotropic interactions. Ideally it is 1.

To conclude, RCSAs deliver orientation information that can be used to determine the configuration of molecules. For estrone, we could show that ΔRDCs and ΔΔRCSAs together, but not individually, allowed a clear differentiation from 13-*epi*-estrone. We have introduced a robust way to measure ΔΔRCSAs based on adjustment of two alignments in the NMR tube, using the stretching apparatus, referencing to a carbon atom of the molecule and using differences of ab initio computed CSA tensors for the back-calculation. Further means to change alignment in the same sample tube will be beneficial for this approach, provided that the sample composition is not changed.[13] The combined use of ΔΔRCSAs and ΔRDCs is beneficial, as the number of experimental parameters increases while the number of parameters to be fitted stays constant at five. We expect that RCSAs will be measured and used in the future whenever RDCs are measured to improve the determination of conformation and configuration of small molecules.

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