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Conformational Analysis of an Isoquinolinium Hydrochloride in Water Using Residual Dipolar Couplings

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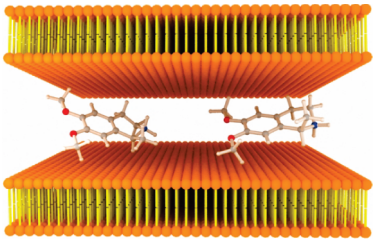

The use of the cetylpyridinium chloride (CPCL)/NaCl/hexanol liquid crystal allowed the measurement of $^{1}D_{CH}$ residual dipolar couplings of the isoquinoline alkaloid salsolidine in its protonated state. Populations of its two half-chair forms were determined by using the single alignment tensor approximation combined with global superposition of conformers. These populations were in good agreement with the DFT-computed energies for both conformers.

Residual dipolar couplings (RDCs) are becoming a powerful complement to classical tools such as NOE and $^{3}J_{HH}$ couplings for stereochemical studies of small organic molecules in solution.[1] Although the use of RDCs in rigid molecules[2] or systems with a predominant conformation[3] is arguably straightforward, the analysis of conformational ensembles is hampered due to the fact that, in principle, the alignment tensor is conformationally dependent. Early approaches to the problem, mostly applied to the analysis of continuous torsional rotation, were based on the additive potential (AP),[4] the maximum entropy (ME),[5] or the hybrid APME models. However, both the AP and ME methods present important shortcomings, as the AP model requires a priori knowledge of the potential surface and the ME methodology furnishes too flat distributions for systems with low orientational ordering. These problems are alleviated by the hybrid APME approach.[6] In general, application of these methodologies to more complex systems with several degrees of freedom is cumbersome. The problem can be greatly simplified assuming (i) a jumping model between stationary points of the potential surface and (ii) independence of the alignment tensor with respect to internal mobility. Based on this single-tensor approach, RDCs have been used as additional restraints in NOE-J restrained molecular dynamics[7] and also in stand-alone procedures.[8] However, the use of a single tensor introduces the additional question of defining the orientation of the different conformers with respect to a common reference frame. Based on a preliminary suggestion by de Lange and Burnell,[9] Thiele and co-workers recently used the Eckart[10] transformation of atomic coordinates to superimpose conformers, thus decoupling the global rotational movement from internal conformational mobility.[11] Note, however, that the Eckart transformation is mathematically equivalent[12] to atomic coordinates least-squares superimposition of conformations in mass weighted coordinates, and therefore, the difference with Cartesian coordinates superimposition[13] will be rather small for molecules with a homogeneous mass distribution.

In this paper, we address the applicability of RDCs to the determination of the conformational mobility of cyclic ammonium ions in solution. Since amines are mostly protonated at physiological pH, the conformational analysis of these ammonium ions in aqueous solution is much more relevant to their biological activity than the analysis of the free amines in organic solvents. The CPCL/NaCl/hexanol lamellar phase[14] is a well-known alignment medium, exten-

(1) (a) Thiele, C. M. *Eur. J. Org. Chem.* **2008**, 5673. (b) Kummerlöwe, G.; Luy, B. *TrAC, Trends Anal. Chem.* **2009**, *28*, 483. (c) Kummerlöwe, G.; Luy, B. *Annu. Rep. NMR Spectrosc.* **2009**, *68*, 193.
(2) (a) Thiele, C. M. *J. Org. Chem.* **2004**, *69*, 7403. (b) Thiele, C. M. *Concepts Magn. Reson. Part A* **2007**, *30A*, 65.
(3) (a) Gil, R. R.; Gayathri, C.; Tsarevsky, N. V.; Matyjaszewski, K. *J. Org. Chem.* **2008**, *73*, 840. (b) García, M. E.; Pagola, S.; Navarro-Vázquez, A.; Philips, D. D.; Gayathri, C.; Krakauer, H.; Stephens, P. W.; Nicotra, V. E.; Gil, R. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 5670.
(4) Emsley, J. W.; Luckhurst, G. R.; Stockley, C. P. *Proc. R. Soc. London A* **1982**, *381*, 117.
(5) Catalano, D.; Di Bari, L.; Veracini, C. A.; Shilstone, G. N.; Zannoni, C. *J. Chem. Phys.* **1991**, *94*, 3928.
(6) (a) Stevensson, B.; Landersjö, C.; Widmalm, G.; Maliniak, A. *J. Am. Chem. Soc.* **2002**, *124*, 5946. (b) Stevensson, B.; Sandström, D.; Maliniak, A. *J. Chem. Phys.* **2003**, *119*, 2738.
(7) (a) Klages, J.; Neubauer, C; Coles, M.; Kessler, H.; Luy, B. *ChemBioChem* **2005**, *6*, 1672. (b) Reinscheid, U.; Farjon, J.; Radzom, M.; Haberz, P.; Zeeck, A.; Blackledge, M; Griesinger, C. *ChemBioChem* **2006**, *7*, 287. (c) Farès, C.; Hassfeld, J.; Menche, D.; Carlomagno, T. *Angew. Chem., Int. Ed.* **2008**, *47*, 3722. (d) Schuetz, A.; Murakami, T.; Takada, N.; Junker, J.; Hashimoto, M.; Griesinger, C. *Angew. Chem., Int. Ed.* **2008**, *47*, 2032.
(8) (a) Thiele, C. M.; Marx, A.; Berger, R.; Fischer, J.; Biel, M.; Giannis, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 4455. (b) Sánchez-Pedregal, V.; Santamaría-Fernández, R.; Navarro-Vázquez, A. *Org. Lett.* **2009**, *11*, 1471.
(9) Burnell, E. E.; de Lange, C. A. *J. Magn. Reson.* **1980**, *39*, 461.
(10) Eckart, C. *Phys. Rev.* **1935**, *47*, 552.
(11) Thiele, C. M.; Schmidts, V.; Böttcher, B.; Louzao, I.; Berger, R.; Maliniak, A.; Stevensson, B. *Angew. Chem., Int. Ed.* **2009**, *48*, 1.
(12) Kudin, K. N.; Dymarsky, A. Y. *J. Chem. Phys.* **2005**, *122*, 224105.
(13) Kabsch, W. *Acta Crystallogr.* **1976**, *A32*, 922.
(14) Prosser, R. S.; Losonczi, J. A.; Shiyanovskaya, I. V. *J. Am. Chem. Soc.* **1998**, *120*, 11010.

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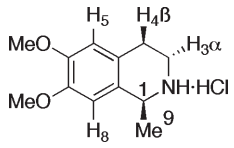

FIGURE 1. Salsolidine hydrochloride **1**.

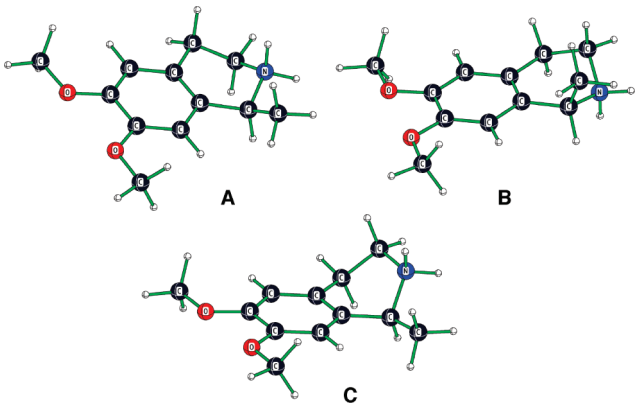

FIGURE 2. Geometries of the three low-energy conformers of salsolidine minimized at the DFT level.

sively used for the measurement of RDC of proteins and oligosaccharides.[15] As this alignment medium is affordable, easy to prepare, and stable over long periods of time, we decided to test its applicability as an alignment medium for protonated amines. As target molecule we chose salsolidine (**1**) (1-methyl-6,7-dimethoxy-2,3,4,5-tetrahydroisoquinoline), an endogenous cyclic amine that displays parkinsonism-preventing properties (Figure 1).[16] Early NMR work on salsolidine ammonium ion showed that this molecule experiences fast conformational exchange, as revealed by its averaged chemical shifts and scalar couplings, a fact that was attributed to the presence of two half-chair conformations.[17] MM3 molecular mechanics exploration of the conformational space of **1** furnished three stationary points (two half-chair forms **1A** and **1B** and a half-boat form **1C**, Figure 2). All these structures were then minimized at the DFT level of theory. According to these computations, the two half-chair forms **1A** and **1B** are very close in energy, and the half-boat conformer **1C** lies 1.9 kcal/mol over the chair forms (Table 1), confirming the previous interpretation of the NMR spectra in terms of a two-site exchange.

RDCs from a 200 mM solution of racemic **1** were extracted from the difference in C−H couplings in an isotropic ($^1J_{CH}$) and anisotropic ($^1J_{CH} + {}^1D_{CH}$) sample. Deuterated water was used as the isotropic medium and the CPCL/NaCl/hexanol liquid crystal as the anisotropic one. C−H couplings were extracted from gated-decoupled ^{13}C spectra. All available one-bond C−H couplings were extracted except those of the methoxy groups, as these groups present extra mobility. We verified that **1** was indeed in its protonated state by successively adding 1 equiv of NaOH and excess HCl to the D_2O solution. Whereas adding the base caused significant changes in the ^1H and ^{13}C chemical shifts due to deprotonation of the N atom, the original values were recovered after addition of the acid (see the Supporting Information).

TABLE 1. Relative Enthalpies of the Three Low Energy Conformations Found in the Conformational Search, Quality Factors (Q), and Condition Numbers (c.n.) of the SVD Fit of Each Conformer

structure	ΔH_0 (kcal/mol)	Q	c.n.
1A	0	0.205	11.08
1B	0.1	0.176	15.49
1C	1.9	0.112	24.82
ensemble			
1A + **1B** (50:50)		0.045	13.20

Initially, each of the conformers was fit separately to the experimental RDCs using the singular value decomposition (SVD) approach.[18] The averaged $^1D_{CH}$ RDC from the freely rotatable methyl group 9 was included in the SVD calculation as described previously.[8b] The methylenic H3β and H3α protons were assigned on the basis of the observation of a NOESY cross-peak between Me9 and H3β. As their corresponding $^1D_{CH}$ values cannot be assigned from the ^{13}C-gated decoupled experiment, they were distinguished from the F2-coupled $^1H-^{13}C$ HSQC spectrum, the largest coupling corresponding to the C3−H3β vector. However, no assignment was possible for the overlapped benzylic H4 protons; hence, only their average $^1D_{CH}$ coupling was taken into account in the SVD procedure by averaging the corresponding entries in the matrix expansion, in a way similar to the procedure used for $^1D_{CH}$ couplings from the methyl group,[8b] thus leaving a total of seven experimental RDC data. The robustness of the SVD procedure is expressed by the condition number (Table 1), i.e., the ratio between the largest and smallest singular values in the SVD. The larger the condition number, the more sensitive the computed alignment tensor is to variations in the experimental RDCs. The goodness of fit between experimental and back-computed RDCs was expressed in terms of the Cornilescu quality factor Q.[19] The relatively large values of the Q factors (Table 1) reflect that none of the conformers fits well to the experimental data, suggesting that there may be more than one conformer contributing to the experimental RDCs. Conformational averaging was in fact expected from the averaged values of chemical shifts and ^3J couplings in the proton spectrum.

In the multiple conformer analysis of RDC, the orientation of each conformer is described, in principle, by an alignment tensor, each requiring five independent RDC values to be determined. Due to the paucity of our RDC data (we had seven experimental RDC values), multitensor fitting is not possible. Since conformational change in salsolidine results in a small perturbation of the overall molecular shape (see Figure 2), we think that single tensor approximation is a valid approach in this case. Given that the C5−H5 and C8−H8 vectors on the benzene ring are nearly parallel, a total of six $^1D_{CH}$ are independent. As the alignment tensor

(15) (a) Skora, L.; Cho, M.-K.; Kim, H.-Y.; Becker, S.; Fernández, C. O.; Blackledge, M.; Zweckstetter, M. *Angew. Chem., Int. Ed.* **2006**, *45*, 7012. (b) Landersjö, C.; Hoög, C.; Maliniak, A.; Widmalm, G. *J. Phys. Chem. B* **2000**, *104*, 5618. (c) Martin-Pastor, M.; Bush, C. A. *J. Biomol. NMR* **2001**, *19*, 125. (d) Lycknert, K.; Maliniak, A.; Widmalm, G. *J. Phys. Chem. A* **2001**, *105*, 5119.

(16) Abe, K.; Saitoh, T.; Horiguchi, Y.; Utsunomiya, I.; Taguchi, K. *Biol. Pharm. Bull.* **2005**, *28*, 1355.

(17) Karimov, M.; Levkovich, M. G.; Leont'ev, V. B.; Sadykov, A. S.; Aslanov, Kh. A.; Yunusov, T. K.; Sadykov, A. A. *Chem. Nat. Compd.* **1974**, *10*, 490.

(18) Losonczi, J. A.; Andrec, M.; Fischer, M. W. F.; Prestegard, J. H. *J. Magn. Reson.* **1999**, *138*, 334.

(19) Cornilescu, G.; Marquardt, J. L.; Ottiger, M. *J. Am. Chem. Soc.* **1998**, *120*, 6836.

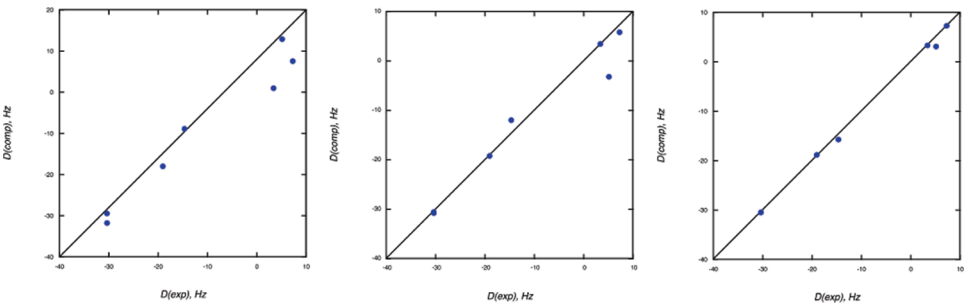

FIGURE 3. Plots of back-calculated vs experimental RDCs after the single tensor analysis. Fit of the two-conformer ensemble (right) is better than that of any of the separate conformers **1A** (left) or **1B** (middle) alone.

has five unknowns, populations of a two-membered ensemble can be computed. As the **1C** form can be safely discarded due to its high DFT computed energy, populations of the two relevant conformers of salsolidine hydrochloride can be determined with the method here proposed.

An assumption on the relative orientation of the conformers has to be made in order to apply the single tensor approximation. We superimposed the two half-chair forms **1A** and **1B** using a least-squares minimization procedure, meaning that structures would be minimally moved with respect to a common external frame. For this least-squares superimposition, we employed a previously described SVD-based algorithm[20] using the coordinates of all heavy atoms.

First, we used a two-conformer ensemble composed by the two half-chair forms **1A** and **1B**, as they are the lowest-energy structures according to our initial conformational search. Data were fit as described previously,[8b] resulting in a better fit than that of either of the separate conformers (Figure 3). A 50:50 ratio was obtained for the two conformations, in good agreement with the DFT calculation that predicts a similar energy for them (Table 1), and with the $^3J_{HH}$ couplings between the C3/C4 protons. Computed $^3J_{HH}$ values using the Haasnoot−Altona equation[21] as implemented in MSpin are shown in Table 2 for each conformer. Lineshape analysis of the proton spectrum gave values in the range of 6.2−6.5 Hz for all four $^3J_{HH}$ couplings, which indicates extensive conformational averaging.

To test the impact of experimental errors, we followed a Montecarlo bootstrapping approach as described previously.[8b] Briefly, experimental RDC values were randomly changed following a Gaussian distribution and the 250 data sets thus created were fit following the same procedure as with the genuine experimental set. As a conservative estimate, we used a standard deviation of 1.5 Hz for each of the measured RDCs. All of the 250 simulations gave consistently similar values for conformer populations, alignment tensors and quality factors Q, indicating that the result is not very sensitive to experimental uncertainty. On average, population of conformer **1A** was 49.5% (standard deviation, s.d., 4.2%), and the $\langle Q \rangle$ factor was 0.056 (s.d. 0.022). Averaged $^3J_{HH}$ couplings using these populations fit well with the observed experimental values (Table 2).

There is no appreciable change of the ^{13}C chemical shifts (see the Supporting Information) between the isotropic and anisotropic conditions, indicating that there is not a significant modification of the conformational populations. Such change would be principally revealed by a displacement of the chemical shift of C3, given that the 1,3-diaxial interaction[22] in **1B** shields the C3 carbon by ca. 5 ppm according to OPBE/6-311+G** GIAO computations.

TABLE 2. Computed Haasnoot−Altona $^3J_{HH}$ Couplings of the Different Conformations and RDC Ensembles

structure	$J_{3\beta-4\beta}$	$J_{3\beta-4\alpha}$	$J_{3\alpha-4\beta}$	$J_{3\alpha-4\alpha}$
1A	5.9	1.2	12.3	3.6
1B	3.8	12.2	1.2	6.1
ensemble				
1A (50%) + **1B** (50%)	4.9	6.7	6.7	4.9
experimental[a]	6.3	6.2	6.5[b]	6.2[b]

[a]Derived from the 1H spectrum by line shape analysis with SpinWorks. [b]H4β and H4α were not assigned.

In conclusion, we have shown that the CPCL/NaCl/hexanol lamellar phase can be used as an anisotropic medium for the RDC-based stereochemical analysis of cyclic amines in their protonated state. Using the simple ^{13}C gated-decoupled experiment, $^1D_{CH}$ RDCs were extracted and conformational probabilities determined, which were in good agreement with high-level DFT computational data.

Experimental Section

The CPCL liquid crystal was prepared with 1 g of D_2O (99.9%); 1.16% (w/w) NaCl; 2.63% (w/w) cetylpyridinium chloride (CPCL); and 2.5% (w/w) *n*-hexanol. All reagents were used as purchased without further purification. Procedure: NaCl (11.6 mg) was dissolved in D_2O (1.0 g) in a magnetically stirred glass vial. When all the NaCl was dissolved, CPCL (26.3 mg) was added with vigorous stirring, and the mixture was heated at 70 °C for 6 min. The mixture was cooled to rt, and then the *n*-hexanol (25 mg) was added and the mixture was heated again at 70 °C for several minutes. Stirring was maintained all the time.

NMR experiments were recorded on a 500 MHz spectrometer equipped with a 5 mm 500 MHz ID/PFG probe (50−202 MHz). The deuterium quadrupolar splitting of the solvent was checked before and after recording the ^{13}C gated-decoupled and F2-coupled HSQC spectra to assess the integrity of the sample. Samples were stable over several days at rt, the deuterium quadrupolar splitting being constant at 18.5 Hz. Spectra were processed and analyzed with the MestReNova software.[23] Phase-sensitive NOESY in D_2O was recorded with a mixing time of 500 ms. RDC analysis was performed using an in-house modified version of the MSpin program.[23]

(20) Mackay, A. L. *Acta Crystallogr.* **1984**, *A40*, 165.
(21) Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783.
(22) Beierbeck, H.; Saunders, J. K. *Can. J. Chem.* **1976**, *54*, 2985.
(23) http://www.mestrelab.com (accessed Feb 1, 2010).

The conformational space of **1** was explored by using the MM3[24] force field and the stochastic search procedure as implemented in PCMODEL.[25] The so-obtained conformations were then minimized at the DFT level of theory using the M052X[26] meta-GGA-hybrid functional and the 6-31+G** basis set. Solvation was taken into account by using the Onsager model[27] with a solvation radium of 4.95 Å and a water relative dielectric constant $\varepsilon = 78.39$. GIAO[28] chemical shifts were computed using the GGA OPBE[29,30] functional and the 6-311+G** basis set on M052X structures and referenced to tetramethylsilane. All DFT computations were performed with the Gaussian03 package.[31]

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra. Coordinates of conformers **1A**−**C**. Tables of experimental and back-calculated RDCs, alignment tensor characteristics, and output files of the SVD fit. Graphical representation of alignment tensor orientation. Lineshape analysis details for determination of $^{3}J_{HH}$ couplings. Complete citation for Gaussian03 program and computed energies and chemical shifts. This material is available free of charge via the Internet at http://pubs.acs.org.

(24) (a) Allinger, N. L.; Yuh, Y. H.; Lii, J. H. *J. Am. Chem. Soc.* **1989**, *111*, 8551. (b) Lii, J. H; Allinger, N. L. *J. Am. Chem. Soc.* **1989**, *111*, 8566. (c) Lii, J. H.; Allinger, N. L. *J. Am. Chem. Soc.* **1989**, *111*, 8576.
(25) *PCModel*; Serena Software: Bloomington, IN.
(26) Zhao, Y.; Shultz, N. E.; Truhlar, D. G. *J. Chem. Theory. Comput.* **2006**, *2*, 364.
(27) Wong, M. W.; Wiberg, K. B.; Frisch, M. J. *J. Am. Chem. Soc.* **1992**, *114*, 523.
(28) Wolinski, K.; Hinton, J. F.; Pulay, P. *J. Am. Chem. Soc.* **1990**, *112*, 8251.
(29) (a) Cohen, A. J.; Handy, N. C. *Chem. Phys. Lett.* **2000**, *316*, 160. (b) Perdew, J. P.; Burke, K.; Ernzerhof, M. *Phys. Rev. Lett.* **1996**, *77*, 3865.
(30) Zhang, Y.; Wu, A.; Xu, X.; Yan, Y. *Chem. Phys. Lett.* **2006**, *421*, 383.
(31) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; et al. *Gaussian03, Revision E.01*; Gaussian, Inc.: Wallingford, CT, 2004.