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Absolute Configuration of Remisporines A & B

Edward C. Sherer* a, James R. Cheeseman*b, R. Thomas Williamsonc

The absolute configuration of remisporine B was determined based on a comparison of experimental and calculated electronic circular dichroism (ECD) spectra. Density functional theory (DFT) was used to calculate the ECD spectra varying the parameter controlling the number of calculated electronic transitions. Mapping the reaction surface provided support for the proposed Diels–Alder dimerization of remisporine A to form remisporine B.

Introduction
Remisporine A (1, Scheme 1), is a fungal metabolite originally isolated from a liquid culture of the marine fungus Remispora maritima.1 Remisporine A was found to be unstable and was shown to autocatalytically dimerize in solution to produce remisporine B, a stereospecific product, via a presumed Diels–Alder reaction. The structure of remisporine B was originally characterized by detailed NMR studies that also revealed its relative stereochemistry (2, Scheme 1).1 An electronic circular dichroism (ECD) spectrum was obtained but at the time of the original work, technology to develop an accurate calculated spectrum for determining the absolute configuration did not exist and the molecule was arbitrarily assigned as shown (2) in Scheme 1. In this work, we show that modern density functional theory (DFT) methods can construct a reliable and satisfying match to the experimental data that assigns the absolute configuration (3, Scheme 1). In addition, we show that an overall exothermic cascade coupled with a reasonable Diels-Alder cyclization barrier supports the proposed mechanism for formation of remisporine B.

Methods
DFT calculations were performed using Gaussian09.2 Conformational space was exhaustively sampled using three conformer generators (rules-based generation relying on sampling if favorable torsion profiles and random displacement which does not consider favorable torsions during generation but for which subsequent energy minimizations are required) followed by molecular mechanics minimization using MMFF94, a workflow that has been previously published.3 DFT with the B3LYP functional4 and the 6-31G** basis set5-7 was used to identify the lowest energy conformers contributing to the Boltzmann distributions for each structure at 298.15 K. All stationary points were confirmed with frequency calculations. To calculate ECD spectra, B3LYP geometries were used as input for calculations using the 6-31++G** basis set either in vacuo or in an implicit solvent using Cramer and Truhlar’s SMD continuum solvation methodology.
The measured ECD spectrum for molecule 2 was calculated using molecular dynamics simulations run with Desmond. An explicit solvent box of methanol was used according to default parameters and a 50 ns simulation was run within the NPT ensemble. Hydrogen bonded solvent molecules were monitored over the course of the trajectory. Snapshots output from the trajectory representing the maximum first shell solvation were used as input for DFT ECD spectra calculations. The explicit complexes were minimized both in vacuo and in implicit methanol.

Characterization of the proposed reaction mechanism was performed using the M062X density functional and the 6-31+G** basis set. All stationary points and transition structures were confirmed with frequency calculations (single imaginary vibration for transition states, and no imaginary vibrations for minima). 

We used a $\sigma = 0.16$ or 0.30 eV for band broadening in this work. Final weighting of the conformer specific ECD spectra was done using the calculated Boltzmann population. Calculation of ECD spectra for molecule 2 required substantial computational resources and long compute times. Increasing the number of electronic states calculated leads to increased computational expense, and owing to limitations in resources, the number of states (NSTATES=20) is commonly set to $\leq 20$. With a smaller number of calculated states caution must be used when assigning absolute configuration since it can be possible to shift the calculated spectrum (of either enantiomer) along the wavelength axis such that the experimental spectrum might match either enantiomer equally well. This arises from the need to commonly shift or scale the calculated frequency range to optimally match experiment. Here we note that a more complete (filling in at lower wavelengths) calculated ECD spectrum can remove this ambiguity.

In vacuo conformer searches led to four predominant conformations for 2 as depicted in Figure 2. The Boltzmann-weighted spectrum is generated from a linear combination of the four calculated ECD curves. Initial matching of the spectra using NSTATES=20 indicated poor confidence in the absolute configuration assignments (Figure S1, Supporting Information). Smoothing of the discreet calculated peaks using band broadening provides the overall ECD spectrum; this smoothing is governed by the $\sigma$ factor set to a default value of 0.16. Increasing $\sigma$ to a value of 0.30 leads to a more smooth surface which better approximates the curvature of the measured spectrum. Smoothing accounts for conformational diversity, solvent effects, and artifacts in geometry arising from assuming ground state geometries for the excited states. Discreet peaks allow for an easy comparison of spectra generated from different computational methods or when comparing spectra and intensities derived from increasing the number of sampled electronic transitions. Figure S1 provides the in vacuo and implicit methanol calculated ECD using NSTATES=20, 50, and 100 states. Since the experimental spectrum went down to 200 nm, it was not until rather higher values of NSTATES was used that the entire measured spectrum was reproduced computationally. Increasing the number of calculated transitions to better approximate the full range of an experimental ECD has been reported.

Figure 1 shows the measured ECD spectrum for 2 or 3 measured in methanol. While the assignment of relative configuration was made by NMR, assignment of the absolute configuration by Kong and Carter was arbitrary. The published spectrum and the mirrored enantiomeric spectrum are overlaid. Calculation of the ECD spectrum for the two enantiomers should allow for unambiguous assignment of absolute configuration.
A small number of calculated electronic states initially populates lower energy transitions which have longer wavelengths, and in order to reach to the lower end of the experimental spectrum at 200 nm, the electronic transitions are higher in energy and for this reason the number of states must be increased significantly. Owing to the large size of the molecule, there are a large number of electronic transitions relative to smaller organic molecules routinely analyzed using ECD. In moving from 20 to 100 states, the distribution of transitions at lower wavelength increases, and dramatically influences the relative intensities of dominant peaks in the spectrum leading to better agreement in the match to experiment. The most dominant feature in the spectrum is the peak at approximately 203 nm which is an $n \rightarrow \pi^*$ transition from the cyclopenta[b]chromen-9(1H)-one ketone oxygen of the ring to the opposite cyclopenta[b]chromen-9(1H)-one ring, and is not identified until excited state 61 out of 100.

When the calculated ECD spectra are overlaid (Figure 3) onto the published ECD for proposed enantiomer 2 or enantiomer 3, it is apparent that the assigned configuration should be (RRRSS) as depicted in Scheme 1 for 3 and not (SSRRR) for 2 as originally proposed by Kong and Carter. A comparison of the $in\ vacuo$ ECD spectra and the implicit methanol spectra indicates good agreement.

Since there is a significant concentration of hydrogen bond donors on 2, we pursued the identification of a solvated complex to determine the effect of explicit solvation on the calculated ECD. Molecular dynamics simulations were run in explicit methanol to determine optimal first shell solvation. The number of directed hydrogen bonds between solvent and 2 over the course of the trajectory peaked at nine. For this reason, diverse snapshots were extracted from the trajectory where nine solvent molecules were coordinated to 2. These snapshots were minimized with B3LYP and energetic comparison led to one dominant solvated structure depicted in Figure 4.

Figure S2 compares the calculated ECD for the $in\ vacuo$ explicitly solvated complex, and the explicit solvent complex further minimized within implicit methanol (both calculated using NSTATES=100). Increasing NSTATES to 200 for the $in\ vacuo$ explicit solvent complex led to an identical calculated ECD as that for NSTATES=100 (data not shown).

Calculated ECD derived from modelling 2 $in\ vacuo$, using implicit methanol, adding explicit methanol solvation, or modelling the explicit complex in implicit methanol were all in good agreement.

The proposed reaction mechanism (Scheme 2) was investigated with the M062X density functional using a moderately sized basis set 6-31+G** and B3LYP/6-31G**. Publications by
Houk and Cramer indicate that these DFT methods should be sufficient for interpreting Diels-Alder transition states and energetics. Free energy values included in the following text are M062X/6-31+G** (see Supporting Information for details and B3LYP numbers). The Diels-Alder reaction is calculated to have an activation free energy barrier (in vacuo) of 15.2 kcal/mol with an exothermic free energy of reaction moving from 1 to 4 of -29.0 kcal/mol. The reaction is slightly endothermic, moving through the retro-Aldol conversion to 5 at 5.8 kcal/mol. Aromatization from 5 to 6 is exothermic at -23.2 kcal/mol, with the final cyclization to form 2 being endothermic by 2.2 kcal/mol.

The transition state for the proposed cyclization of 1 to 4 is provided in Figure 5. A calculated barrier of ~15 kcal/mol for the initial Diels-Alder cyclization is quite low, and well within the normal ranges for this type of reaction (e.g., the Diels-Alder closure of butadiene + ethylene is 27.5 kcal/mol[6,6]). Electronic reaction energies calculated with B3LYP are similar to the M062X values, and owing to little charge build up in the transition state, implicit solvation did not change the B3LYP barrier height significantly (ΔG difference of -1.5 kcal/mol). We attribute a rather large difference between the free energy barriers calculated using B3LYP and M062X to the better treatment of dispersion effects which are significant in the stacked pre-transition state complex and the transition state itself (leading to a difference of ~10-12 kcal/mol). These differences were further investigated by mapping the first step in the proposed mechanism using three additional DFT methods: CAM-B3LYP[7] long range correction, B3LYP with D2[8] with dispersion correction, and D3[9] dispersion and damping corrections (Supporting Information). With a better treatment of dispersion effects, the D2 and D3 corrected energies show favorable energetics of forming the van der Waals complex similar to M062X.

Determining that the proposed Diels-Alder reaction pathway is reasonable does not determine absolute configuration, however, Diels-Alder reactions are known to impart a high degree of stereo- and regio-selectivity. While a series of calculations to gauge the facial and endo/exo selectivity of the Diels-Alder step is of interest and would lend support to the final relative configuration, the computational cost limits our further pursuit of these calculations.

Figure 5: Transition state for the Diels-Alder cyclization of 1 to 4 calculated at the M062X/6-31+G** level (SSRRR).

Conclusions
Calculation of ECD spectra has indicated that the absolute configuration depicted for 2 in the original publication of the structure of remisporine B needs to be revised. By explicitly calculating the ECD spectra for 2 and 3 we have shown that the absolute configuration of remisporine B is (RRSSS) as depicted in Scheme 1 for 3. The overall exothermic nature of the proposed reaction mechanism and the reasonable barrier to Diels-Alder cyclization is consistent with the originally proposed mechanism for the autocatalytic dimerization of remisporine A to form remisporine B. Based on these conclusions, the actual absolute stereochemistry of remisporine A (7, Scheme 3) should be assigned accordingly as S.

Notes and references
2. M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B.

Scheme 2

Scheme 3
17. Y. Zhao, and D.G. Truhlar, *Chemistry-a European Journal*, 2011, 17(42), 11868.


