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Relative Configuration of Natural Products Using NMR Chemical Shifts

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Received August 14, 2008

We have measured and quantum chemically computed NMR chemical shifts for three monoterpene diastereomers produced by the walkingstick, *Anisomorpha buprestoides*. By taking into account the Boltzmann distribution of conformers, the combined RMSDs between experimental and calculated ¹H and ¹³C NMR shifts were able to determine the correct isomer, especially when only aliphatic nuclei were used. The calculated relative energies and interproton distances were also consistent with chemical isomerization experiments and NOE-based interproton distance calculations. Complementary to the NOE-based method, a comparison between experimental and calculated NMR chemical shifts can provide an efficient method to assign the relative configuration of natural products.

NMR spectroscopy is a powerful tool for natural product discovery,^{1–3} as it can be used to probe a variety of important structural features. Some NMR techniques, such as those that target covalent connectivity, are well developed. Determining the relative configuration of organic molecules, on the other hand, is frequently a difficult task with NMR. Although many NMR parameters, such as chemical shifts,⁴ *J*-coupling constants,⁵ nuclear Overhauser effect (NOE),⁶ and residual dipolar couplings,⁷ are sensitive to differences in relative configuration, these differences among various stereoisomers are often small and not readily interpreted. Another problem often faced in the assignment of relative configuration is that not all possible stereoisomers are available, as the synthesis of optically pure compounds with complicated structures and several stereogenic centers is quite challenging.

NMR chemical shifts are sensitive to the local electronic environment of magnetically active nuclei, which is affected by the relative configuration. Therefore, proton (¹H) and carbon (¹³C) chemical shifts can be a useful tool for distinguishing various diastereomers. However, the interpretation of chemical shifts rarely exceeds the identification of functional groups, and much of the information contained in chemical shifts is not used. Modern developments in quantum chemistry facilitate the calculation of NMR chemical shifts rapidly, accurately, and reliably.⁸ Therefore, a comparison between theoretical and experimental chemical shifts can provide an efficient way to identify and discriminate diastereomers. Wiitala et al.⁹ have demonstrated that computed ¹H and ¹³C chemical shifts using several density functional theories are able to distinguish between *cis* and *trans* stereoisomers for 2-, 3-, and 4-methylcyclohexanols after the Boltzmann averaging of possible conformers. Although this approach is attractive, whether it is successful in determining relative configuration of natural products of more flexible and complicated structures with several stereocenters is still an open question.

Recently, we found that the active component in defensive spray of *Anisomorpha buprestoides*, a walking stick insect common to Florida, consists of varying amounts of three cyclopentanoid

monoterpene diastereomers called dolichodial (**1**), anisomorphal (**2**), and peruphasmal (**3**) (Scheme 1).^{10,11} For simplicity, we will refer to these three isomers collectively as “dolichodial-like”. These compounds, each with flexible functional groups and three stereocenters, provide an opportunity to test high-level theoretical chemical shift calculations for their ability to differentiate between the dolichodial-like diastereomers. This work demonstrates the power of using theoretical calculations based on quantum chemistry to extract the valuable information contained in NMR chemical shifts and thus provides a novel approach to assign the relative configuration of natural products.

Results and Discussion

Chemical Isomerization. We conducted chemical stability studies by incubating either dolichodial or peruphasmal under acidic, neutral, or basic conditions for up to one week and monitored their stability by GC-FID (data not shown). Dolichodial equilibrated into a mixture of dolichodial (**1**) and anisomorphal (**2**) under either acidic or basic conditions. Peruphasmal (**3**), on the other hand, did not isomerize under any condition. Presumably, the aldehyde f² can form an enol or enolate intermediate and racemize, converting into a mixture of dolichodial and anisomorphal (Scheme 1). A similar racemization with peruphasmal would result in the placement of all exocyclic groups on the same side of the cyclopentane ring, a configuration that is energetically unfavorable (*vide infra*).

NOE. The NOE protocol provides a qualitative measure of ¹H–¹H distances up to 5 Å through space. This information can be useful for determining the relative configuration of natural products, but conformational averaging, especially in groups such as cyclopentanyl rings, makes quantitative interpretation of NOEs challenging. Table 1 lists some diagnostic experimental distances estimated from NOE volumes for three dolichodial-like molecules from walkingsticks (see Experimental Section) and corresponding calculated theoretical Boltzmann-averaged interproton distances from all four possible diastereomer structures. The short distance between H-1 and H-2 for dolichodial (**1**) indicates its vinyl aldehyde and aldehyde group are at the same side of cyclopentane ring, whereas the relatively large distances for anisomorphal (**2**) and peruphasmal (**3**) suggest these two groups are on opposite sides in both molecules. The NOE intensities of H-2 and H-3 clearly show that those protons are cofacial for anisomorphal, but are on the opposite side for dolichodial and peruphasmal, placing the aldehyde and methyl group on the same side for anisomorphal and on opposite sides for dolichodial and peruphasmal. Other NOE cross-peaks in Table 1 are also characteristic of the relative configuration

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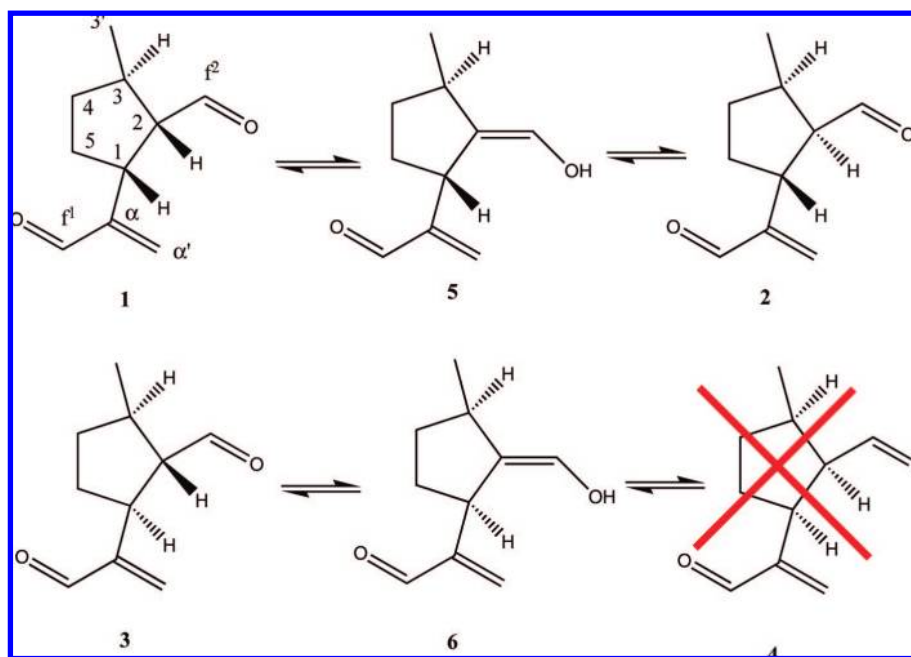
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Scheme 1. Chemical Equilibria of Dolichodial-like Compounds under Acidic or Basic Conditions in Aqueous Solution^a

^a Dolichodial (**1**) equilibrates to anisomorphal (**2**) under acidic or basic conditions. We hypothesize that this happens through an enol/enolate intermediate depending on pH. However, peruphasmal (**3**) does not isomerize under these conditions: the all-*cis* configuration (**4**) has not been observed. This is likely due to steric hindrance preventing the formation of the all-*cis* configuration of the molecule (**4**).

Table 1. Diagnostic ¹H–¹H Distances Estimated from Nuclear Overhauser Effect (NOE) Peaks Observed for the Three Naturally Occurring Diastereomers of Dolichodial from *A. buprestoides*^a

	dolichodial 1	anisomorphal 2	peruphasmal 3	<i>4</i>
H1–H2	2.19	2.29	3.00	<i>2.78</i>
H1–Hf ²		3.83	2.80	<i>2.45</i>
H2–H3		2.98	2.23	<i>2.31</i>
H2–Hα ^b		4.53	2.63	<i>2.45</i>
H3–H5a	2.86	2.77	3.03	<i>3.40</i>
H3–Hf ²	2.77	2.98	3.66	<i>2.50</i>

^a The calculated Boltzmann-averaged interproton distances (in Å) for all possible stereoisomers are also listed for comparison. The distances for **4** are in italics because this compound has not been experimentally observed and its distances do not correlate with observed NOEs. The complete list of experimental NOE distances is provided in the Supporting Information.

Table 2. Correlation Coefficients (*R*²) between the Estimated Interproton Distances from Measured NOE Volumes for the Three Naturally Occurring Diastereomers of Dolichodial-like Compounds from *A. buprestoides* and the Calculated Boltzmann-Averaged Distances for All Possible Stereoisomers^a

	dolichodial	anisomorphal	peruphasmal
1	0.8923	0.5107	0.4199
2	0.5401	0.8202	0.3580
3	0.3463	0.4673	0.8073
4	0.4705	0.5472	0.3105

^a The best agreement for each diastereomer is highlighted in bold type.

of the three molecules. Moreover, the correlation coefficients (*R*²) between distances estimated from NOE volumes and the calculated interproton distances (Table 2 and Supporting Information, Table S1) clearly show that the theoretical distances for **1**, **2**, and **3** match the qualitative NOE pattern for dolichodial, anisomorphal, and peruphasmal, respectively. The structure of **4** does not match any NOE patterns of the three observed compounds. On the basis of these observations, we can tentatively assign computational structure **1** to dolichodial, **2** to anisomorphal, and **3** to peruphasmal.

NMR Chemical Shifts. It is often a challenge to distinguish diastereomers of organic compounds using NMR chemical shifts, because the differences in chemical shift of their corresponding nuclei are often small, as shown in Table 3. The correlation coefficients (*R*²) for all Boltzmann-averaged ¹H and ¹³C chemical shifts for **1**–**4** vs the three experimental data sets (dolichodial, anisomorphal, and peruphasmal) are 0.99 and above (see Table 4). This limits the ability of *R*² values as determinants of the configuration of these compounds. Nevertheless, Table 4 still indicates that, for both ¹H and ¹³C chemical shifts, **2** and **3** have the best agreements with anisomorphal and peruphasmal, respectively. Structure **1** matches dolichodial the best in ¹H chemical shifts and the second best in ¹³C, and the chemical shifts for **4** have the worst agreement with experimental data sets for all three compounds.

We also calculated the root-mean-square deviations (RMSDs) between theoretical and experimental NMR chemical shifts. To improve its ability to distinguish the stereoisomers, we utilized both ¹H and ¹³C chemical shift information by defining combined root-mean-square deviation (cRMSD) as

$$\text{cRMSD} = \text{RMSD}({}^{13}\text{C}) + 10 \times \text{RMSD}({}^1\text{H}) \quad (1)$$

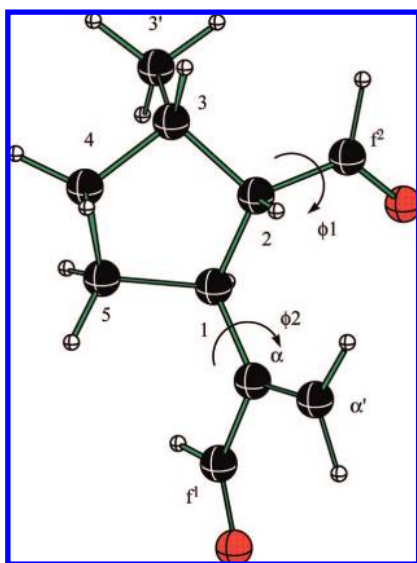
The coefficient 10 is used to normalize the influence of ¹³C and ¹H chemical shifts on the overall RMSD by roughly correcting for the chemical shift ranges of these nuclei. Figure 2 (red bars) shows the cRMSD of **1**–**4** against three sets of experimental data. Clearly, chemical shifts obtained from **1**, **2**, and **3** have the lowest cRMSDs for experimental data in dolichodial, anisomorphal, and peruphasmal, respectively. Structure **4** yields the largest cRMSDs across the three experimental data sets. Moreover, as expected, their respective enantiomers give an almost identical result. Therefore, we can draw the same conclusion as from NOE observations: dolichodial, anisomorphal, and peruphasmal have the structures of **1**, **2**, and **3**, respectively. Compound **4** has not been experimentally observed in the defensive spray of walkingstick insects nor in any other reports. Interestingly, structure **4** has the highest global minimum energy compared to the other three structures (Figure

Table 3. ^1H and ^{13}C NMR Chemical Shifts of Dolichodial (1), Anisomorphal (2), and Peruphasmal (3) in Benzene- d_6 (ppm)^a

position	dolichodial (1)		anisomorphal (2)		peruphasmal (3)	
	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)
1	39.9	3.02, m	38.2	3.28, m	40.1	2.95, m
2	60.2	2.51, m	59.0	2.48, m	64.3	2.01, m
3	34.2	2.23, m	37.2	2.13, m	36.6	1.76, m
3'	20.5	0.81, d (6.8)	16.4	0.72, d (7.2)	19.3	0.81, d (6.7)
4	33.4	0.83, m	34.5	1.02, m	33.4	1.06, m
		1.64, m		1.61, m		1.52, m
5	30.0	1.28, m	30.6	1.29, m	30.5	1.75, m
		1.36, m		1.79, m		1.30, m
α	150.4		152.5		152.0	
α'	133.6	5.26, s	132.9	5.17, s	132.5	5.15, s
		5.54, d (1.1)		5.60, s		5.62, d (0.9)
f ¹	193.7	9.11, s	193.7	9.12, s	193.5	9.09, s
f ²	202.1	9.25, d (2.4)	202.8	9.48, d (2.8)	201.5	9.26, d (4.1)

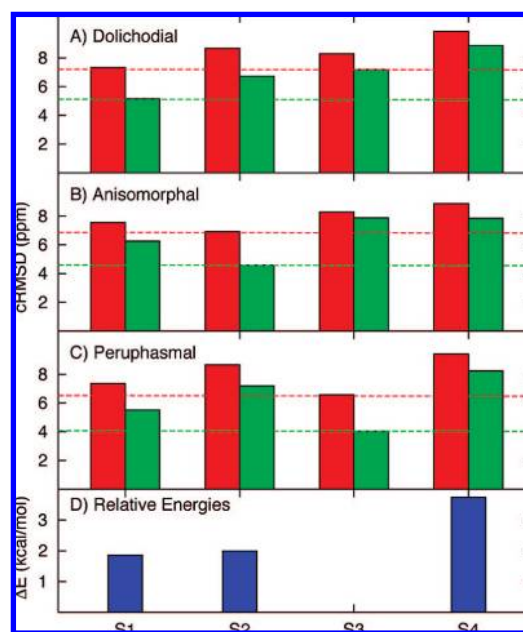
^a All spectra were referenced by setting the benzene- d_6 chemical shifts to $^1\text{H} = 7.16$ ppm and $^{13}\text{C} = 128.39$ ppm.**Table 4.** Correlation Coefficients (R^2) of Calculated NMR Chemical Shifts of All Nuclei from Possible Dolichodial-like Diastereomers against Experimental Values from Three Naturally Occurring Compounds^a

	dolichodial		anisomorphal		peruphasmal	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1	0.9936	0.9961	0.9899	0.9959	0.9936	0.9962
2	0.9946	0.9943	0.9966	0.9963	0.9914	0.9956
3	0.9898	0.9956	0.9872	0.9959	0.9972	0.9968
4	0.9903	0.9927	0.9915	0.9935	0.9935	0.9936

^a The best agreements are highlighted in bold type.**Figure 1.** Structure and numbering of dolichodial-like molecules and rotatable bonds for chemical shift calculations. The numbering scheme is according to Chemical Abstracts Service.

2D) because of steric constraints in this structure. Most importantly, this is also consistent with the experimental observations.

The above analysis is based on all ^1H and ^{13}C NMR chemical shifts of dolichodial-like cyclopentanoid monoterpene dialdehyde molecules. However, it is reasonable to consider only the aliphatic ^1H and ^{13}C nuclei because (a) most of atoms around the stereocenters are aliphatic; (b) the systematic errors associated with the computational method are quite different with various types of nuclei, and it has been shown that B3LYP/6-311++G** is able to predict aliphatic proton chemical shifts with high accuracy;¹² and (c) these monoterpenes contain six aliphatic carbon and 10 aliphatic hydrogen atoms (eight of them are NMR distinguishable), which provides a statistically significant set of data points for analysis. Table 5 lists the correlation coefficients of calculated vs experi-

**Figure 2.** cRMSD values for NMR chemical shifts of all (red) and aliphatic (green) nuclei for each computed isomer (Scheme 1, 1–4) compared to experimental data for dolichodial (A), anisomorphal (B), and peruphasmal (C). The dashed red and green lines are drawn at the height of best agreement for each compound. (D) Relative global minimum energies for 1–4.**Table 5.** Correlation Coefficients (R^2) of Calculated NMR Chemical Shifts of Aliphatic Nuclei from Possible Dolichodial-like Diastereomers against Experimental Values from Three Naturally Occurring Compounds^a

	dolichodial		anisomorphal		peruphasmal	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1	0.9632	0.9572	0.9431	0.9453	0.9039	0.9461
2	0.9486	0.9191	0.9873	0.9823	0.8540	0.9356
3	0.8555	0.9769	0.8350	0.9699	0.9672	0.9772
4	0.8784	0.8862	0.9275	0.9081	0.8581	0.8924

^a The best agreements are highlighted in bold type.

mental chemical shifts for these nuclei. By comparing it with Table 4, we can draw the same conclusion, but with much higher confidence. For example, the difference between the highest and the second highest R^2 values for ^1H chemical shifts in the anisomorphal set is 0.06 instead of 0.005. The cRMSDs values for aliphatic nuclei are plotted in Figure 2 (green bars). Again, the ability to distinguish stereoisomers significantly increases. For instance, the difference of cRMSD in the anisomorphal set between

2 (the lowest) and 1 (the second lowest) is 1.7 ppm instead of 0.6 ppm for all nuclei.

Conclusions

We have demonstrated the use of NMR chemical shifts to determine the relative configuration of three naturally occurring cyclopentanyl monoterpene dialdehydes. This was accomplished by calculating the potential energy surfaces and then Boltzmann-averaging computed ^1H and ^{13}C NMR chemical shifts for all possible configurations of the three dolichodial-like isomers produced by *A. buprestoides*. Our computed results were then compared to experimental values, allowing us to identify the relative configurations of the diastereomers. All structural assignments were verified by both chemical equilibrium experiments and traditional NMR NOE correlations. These results demonstrate that a combination of computationally derived and experimental NMR chemical shifts can accurately determine the relative configurations of natural products, if taking into account the Boltzmann distribution of conformational isomers. We believe that more examples will prove that chemical shift calculations are a useful complement to experimental techniques in stereochemical analysis for natural products.

Experimental Section

General Experimental Procedures. Dolichodial (**1**) was collected as a single secretion from an immature *A. buprestoides*, which, at the time, produced predominantly that isomer.¹⁰ Anisomorphal (**2**) and peruphasmal (**3**) were obtained as single secretions from adult *A. buprestoides* collected among populations that produce only one or the other of those isomers.¹¹ Secretions were extracted with benzene- d_6 , and experimental NMR chemical shifts were measured and assigned using standard 1D and 2D experiments, as previously described.¹³ All spectra were referenced by setting the benzene chemical shifts to $^1\text{H} = 7.16$ ppm and $^{13}\text{C} = 128.4$ ppm. Experimental NMR chemical shifts and coupling information can be found in Table 3. 2D NOESY data sets were obtained with a two-second mixing time. In order to estimate interproton distances from NOE volumes, we used the following equation:

$$r = \left(\frac{r_{\text{ref}}^6 V_{\text{ref}}}{V} \right)^{1/6} \quad (2)$$

where V and V_{ref} are the volumes of the unknown and reference peak, and r and r_{ref} are the corresponding interproton distances. We used the cross-peak between geminal protons at C-4 as the reference peak since this peak is always present for all three diastereomers and the r_{ref} value is well defined (1.76 Å). The NOE volume V is the average volume of the corresponding cross-peaks from both sides of the diagonal. Direct comparisons (and their corresponding R^2) values between experimental NOE-based calculated distances and theoretical distances calculated by averaging distances among the same sets of conformations used to calculate theoretical chemical shifts can be found in Tables 1 and 2, Supporting Information Table S1, and Supporting Information Figure S1.

Chemical isomerization studies were done by incubating either pure dolichodial or pure peruphasmal in 10 mM acetate buffer at pH 4.5, 10 mM phosphate buffer at pH 7.0, and 10 mM Na_2CO_3 buffer at pH 9.5 for one week. NaCl was added to maintain constant ionic strength ($\mu = 0.1$) across all experiments. As described previously, the reactions were monitored by gas chromatography–flame ionization detection (GC-FID) with retention comparison to authentic standards.

Computational Details. With three stereogenic centers, there are four possible enantiomeric pairs of dolichodial-like diastereomers that we designated **1–4** (Scheme 1). We optimized geometries and calculated all eight enantiomers independently and found, as expected, essentially complete agreement in energies and calculated chemical shifts between pairs of enantiomers. The 2D potential energy surfaces (PES) were computed by rotating the two exocyclic bonds at carbon 1 and 2 in 30° increments (Figure 1), producing 144 conformers for each stereoisomer. Each conformer was optimized at the B3LYP¹⁴/6-31+G*¹⁵ level. The pseudorotation barrier of the cyclopentane ring,

usually below 0.5 kcal/mol, was relaxed during the optimization process. Next, NMR chemical shieldings for each conformer were calculated using gauge-including atomic orbitals (GIAO)^{16,17} at the B3LYP/6-311++G** level, which has been shown to produce accurate NMR chemical shifts.^{12,18,19} To account for solvent effects, we applied the implicit solvent model IEFPCM developed by Tomasi and co-workers.^{20,21} Benzene- d_6 was used as the solvent in the experiments, so we also calculated NMR chemical shieldings for benzene at the same theoretical level as the reference value. The final chemical shifts for each stereoisomer were computed by Boltzmann-weighted averaging for all conformers:

$$\langle \delta \rangle = \frac{\sum_i \delta_i e^{-\Delta E_i/kT}}{\sum_i e^{-\Delta E_i/kT}} \quad (3)$$

where ΔE_i is the relative energy of the i th conformer to the lowest energy, k is the Boltzmann constant, and the temperature T is set to 298 K. For each conformer, the calculated chemical shifts for protons in the methyl group were averaged arithmetically due to their free rotation at ambient temperature. All calculations were carried out by using the Gaussian 03 package under Linux.²²

Acknowledgment. NMR spectra were obtained in the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) facility in the McKnight Brain Institute at the University of Florida. We thank J. R. Rocca for help with NMR. This was supported by a Human Frontier Science Program grant (A.S.E.) and the National High Magnetic Field Laboratory (NSF DMR-0654118). K.M.M. thanks the NSF (MCB-0211639) for supporting this research.

Supporting Information Available: Complete lists of experimental NOE distances and calculated distances, comparison plots of experimental NOE and theoretical distances, and the potential energy surface (PES) for molecules **1–4**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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NP8005056