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Extension of the *J*-Based Configuration Analysis to Multiple Conformer Equilibria: An Application to Sapinofuranone A

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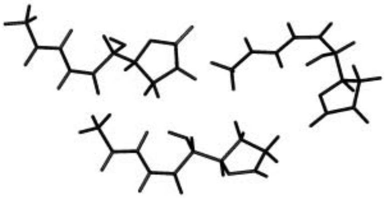
ABSTRACT



A new strategy that extends the application of the *J*-based configuration analysis to systems characterized by multiple conformer equilibria is described and applied to sapinofuranone A (1), a phytotoxic molecule produced by three strains of *Sphaeropsis sapinea*. This method, based on a combination of computational techniques and NMR spectroscopy, uses ab initio calculations to predict a set of theoretical homo- and heteronuclear *J* values which can be compared against experimental NMR data.

One of the crucial points in the structural study of complex organic molecules, notably of bioactive natural products, is the elucidation of their stereochemical features. With this aim in mind, an NMR *J*-based configuration analysis[1] has recently been devised for the stereochemical determination of acyclic structures and has been applied to the study of several natural products.[2−5] The original strategy deals with the combined use of homonuclear and heteronuclear coupling constants ($^3J_{HH}$, $^{2,3}J_{CH}$) and NOE/ROE data, which enables the determination, for a two-carbon fragment, of the predominant rotamer(s) with the correct configuration from among the six possible staggered conformers, since each single rotamer shows its own homonuclear and heteronuclear coupling constant pattern. Recently, our research group has reported two NMR spectroscopy studies on the relative configuration analysis of flexible natural products, in which we applied our own modified[6,7] version of the *J*-based configuration analysis developed by Murata et al.[1] The methodology may be applied in the presence of multiple conformers, provided that at least one or two of the

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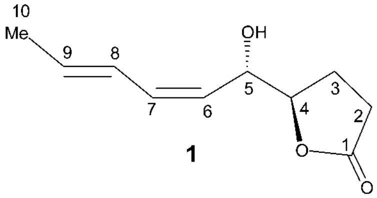
conformers account for 90% of the global population. Nevertheless, the assignment of the relative configuration becomes ambiguous or impossible when the averaging values of the NMR parameters do not allow the full characterization of a conformational equilibrium occurring in a regime faster than the NMR time scale.

We reasoned that this intrinsic limitation in the methodology may be overcome by using ab initio calculations to predict the molecular geometries and relative energies of all relevant conformers of each possible diasteroisomer of the system under investigation. With the latter information available, the computation of theoretical values of crucial NMR parameters can be based on an accurate knowledge of the Boltzmann distribution for all interconverting conformers. Theoretical data for each stereoisomer, such as chemical shifts and *J*-couplings, can be carefully examined against the experimental NMR data for a compound with an unknown configuration, leading, in the case of a satisfactory match for *only one* of the possible diasteromers, to the determination of its relative configuration.

In the course of our ongoing program on NMR stereochemical studies,[8,9] we envisaged the possibility of testing this extended *J*-based approach on the natural product sapinofuranone A (**1**), a flexible molecule characterized by complex multiple conformer equilibria and for which the application of the *J*-based analysis in its original formulation led to conflicting results (see below). Sapinofuranone A (**1**) is a new 5-substituted dihydrofuranone displaying phytotoxic activity produced, along with its C-5 epimer sapinofuranone B, by three strains of *Sphaeropsis sapinea* isolated from infected cypress trees.[10] The molecule possesses two adjacent stereocenters in a 10-carbon skeleton. The absolute configuration at C-5 was determined by applying the Mosher method.[10] Full stereochemical knowledge on **1** and related compounds was subsequently acquired by stereoselective synthesis of *ent*-sapinofuranone B and chemical correlation to other known natural products.[11]



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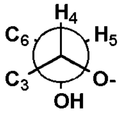
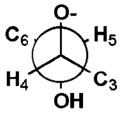
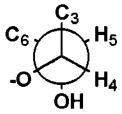
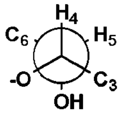
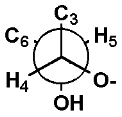
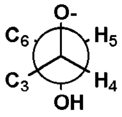
The set of NMR data obtained by applying the plain *J*-based strategy to **1** was unable to identify unambiguously just one of the six possible rotamers of the fragment of interest (C4−C5), namely **X1**, **X2**, **X3** with 4*R**,5*S** relative configuration and **Y1**, **Y2**, **Y3** with 4*S**,5*S** relative configuration (Table 1).

Table 1. Dependence of $^{3}J_{H,H}$ and $^{2,3}J_{C,H}$ on Dihedral Angles in the Six Possible Staggered Rotamers of **1**

rotamer	Newman projection	$^{3}J_{H4H5}$	$^{2}J_{H5C4}$	$^{2}J_{H4C5}$	$^{3}J_{H5C3}$	$^{3}J_{H4C6}$
	X (5*S**, 4*R**)					
X1		small	large	small	large	small
X2		large	large	large	small	small
X3		small	small	large	small	large
	Y (5*S**, 4*S**)					
Y1		small	small	small	small	small
Y2		large	large	large	small	small
Y3		small	large	large	large	large

In fact, the homonuclear coupling constant $^{3}J_{H4H5} = 3.1$ Hz, indicative of a gauche orientation for the protons H4−H5, reduced the possible dominant rotamers to the following four out of the initial six: **X1**, **X3**, **Y1**, **Y3** (Table 1). The $^{2}J_{H4C5} = 1.0$ Hz (small), suggesting an anti relationship between H4 and OH at C5, ruled out the rotamers **X3** and **Y3**, further reducing the possible rotamers to **X1** and **Y1**. However, the remaining heteronuclear coupling constants were not in agreement with the presence of either **X1** or **Y1** as dominant conformers. Even worse, these two heteronuclear *J*-coupling values indicated rotamers with opposite configuration (Table 4), implying that none of the above considered *J* values should be interpreted as resulting from a single dominant conformer. In fact, the small $^{3}J_{H5C3}$ value (2.6 Hz), accounting for a gauche relationship between H-5 and C-3, would lead to conformation Y1, whereas the medium/large $^{2}J_{H5C4}$ value (−3.3 Hz)[12] would point to rotamer **X1** (Table 1). Moreover, no dipolar coupling relative to H3/H3′ and

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Table 2. Calculated $^{3}J_{\mathrm{H,H}}$[24] and $^{2,3}J_{\mathrm{C,H}}$[25] Values for the Conformers Found for the Diastereoisomers **X** and **Y**

conformer	dihedral angle (deg) for the fragment: $H_4C_4C_5H_5$	dihedral angle (deg) for the fragment: $H_5C_5C_6H_6$	$^{3}J_{\mathrm{H4H5}}$	$^{2}J_{\mathrm{H5C4}}$	$^{2}J_{\mathrm{H4C5}}$	$^{3}J_{\mathrm{H5C3}}$	$^{3}J_{\mathrm{H4C6}}$
Xa	+67.4	160.4	2.0	−4.3	3.0	4.0	0.4
Xb	173.8	169.0	8.6	−4.6	−3.3	1.9	3.0
Xc	−62.7	174.8	1.7	3.0	−4.7	0.2	4.9
Xd	175.4	−31.8	8.7	−4.5	−3.3	2.1	2.4
Xe	67.3	−120.3	2.0	−4.3	3.0	4.0	0.5
Ya	70.7	−178.0	0.3	3.0	3.0	1.2	1.8
Yb	178.8	168.5	8.9	−3.9	−3.5	2.0	1.5
Yc	−60.8	158.1	3.8	−4.6	−4.6	4.6	5.1
Yd	−54.5	−97.2	4.6	−4.5	−4.5	5.3	5.1
Ye	−174.8	−22.7	8.4	−4.0	−4.0	1.5	0.9

H5, which might have been useful to distinguish the two rotamers **X1** and **Y1**, was found in the ROESY spectra.[13]

These apparently conflicting NMR data can be explained by assuming that the system under investigation is characterized by a fast interconversion between two or more conformers. In principle, the assignment of the relative configuration to systems with alternating conformers may also be treated by the original methodology proposed by Murata et al.,[1] taking into due account the averaging values of the coupling constants. However, this approach is only applicable if two out of the three possible staggered rotamers for each diastereoisomer are predominant, i.e. the third rotamer must account for less than 10% of the population, its contribution to NMR data therefore being negligible. In the case of sapinofuranone A (**1**), homonuclear and heteronuclear *J* values do not identify any alternating pair of rotamers, rather, they suggest an equilibrium between all three rotamers.

Once we had established that the *J*-based methodology in its original formulation was not suitable for the stereochemical study of **1**, we moved on to test our extended version of the *J*-based approach. The application of the strategy being proposed consisted of four main steps: (a) sampling of the conformational space accessible to each diastereomer for the purpose of identifying the representative conformers for each stereoisomeric series; (b) geometry optimization at the Hartree−Fock level of the conformers thus obtained, followed by the calculation of their heat of formation; (c) determination of the Boltzmann weighting factors of all the representative conformers for each stereoisomer; and (d) computation of the averaged *J*-couplings and then a comparison between calculated and experimental *J* values ($^{2,3}J_{\mathrm{C,H}}$, $^{3}J_{\mathrm{HH}}$).[14−16]

Table 3. Weighted Average $^{3}J_{\mathrm{H,H}}$ and $^{2,3}J_{\mathrm{C,H}}$ Values [26](Hz) for the Diastereoisomers X and Y

conformer	% of the total population	$^{3}J_{\mathrm{H4H5}}$	$^{2}J_{\mathrm{H5C4}}$	$^{2}J_{\mathrm{H4C5}}$	$^{3}J_{\mathrm{H5C3}}$	$^{3}J_{\mathrm{H4C6}}$
Xa	57.0	1.1	−2.4	1.7	2.3	0.3
Xb	22.0	1.9	−1.0	−0.7	0.4	0.4
Xc	13.0	0.2	0.4	−0.6	0.0	0.0
Xd	7.0	0.7	−0.3	−0.2	0.2	0.2
Xe	1.0	0.0	−0.1	0.0	0.0	0.0
total	100	**3.9**	**−3.4**	**0.2**	**2.9**	**0.9**
Ya	53.0	0.2	1.6	1.6	0.6	0.9
Yb	26.0	2.3	−1.0	−0.9	0.5	0.4
Yc	20.0	0.7	−0.9	−0.9	0.9	1.0
Yd	1.0	0.1	−0.1	−0.1	0.1	0.1
Ye	0.0	0.0	0.0	0.0	0.0	0.0
total	100	**3.3**	**−0.4**	**−0.3**	**2.1**	**2.4**

We started with a conformational search on the two **X** (4*R**, 5*S**) and **Y** (4*S**, 5*S**) diastereoisomers by molecular dynamics ($T = 500$ K, CVFF[17] force field, constant dielectric = 4.8) in order to include all possible combinations of the staggered arrangements, and in particular all the conformers characterized by different torsion angles around the $H_4-C_4-C_5-H_5$ and the $H_5-C_5-C_6-H_6$ fragments (*gauche*$^{\mathrm{P}}$ +60°, *anti*$^{\mathrm{A}}$ 180°, *gauche*$^{\mathrm{M}}$ −60°, Table 2). Molecular dynamics simulations followed by energy minimizations were performed with use of the Discover module of Insight II.[17] This search provided us with a total number of 16 relevant conformers, 8 for the diastereoisomer **X** and 8 for the **Y**. The geometry of all the conformers was then optimized ab initio at the HF level, with the 6-31G* basis set,[18] using the

Table 4. Experimental and Calculated *J* Values (Hz) of Sapinofuranone A (**1**)[a]

	exptl *J* values of **1**	calcd *J* values of the two diastereoisomers of **1**: **X**	calcd *J* values of the two diastereoisomers of **1**: **Y**
$^{3}J_{\mathrm{H4H5}}$	3.1 (small)	3.9	3.3
$^{2}J_{\mathrm{H5C4}}$	−3.3 (medium/large)	**−3.4**	**−0.4**
$^{2}J_{\mathrm{H4C5}}$	1.0 (small)[28]	0.2	**−0.3**
$^{3}J_{\mathrm{H5C3}}$	2.6 (small)	2.9	2.1
$^{3}J_{\mathrm{H4C6}}$	<1.0. (small)[29]	0.9	**2.4**

[a] The spectra were recorded on a Bruker DRX600 spectrometer at $T = 298$ K, in $CDCl_3$. Calculated *J* values differing more than 1 Hz with respect to experimental values are displayed in bold.

Gaussian 98W package.[19] The convergence of some of the initial conformers to the same geometry and energy[20] (HF energy) reduced the final number of structures for each diastereoisomer to five (Tables 2 and 3).

Homonuclear[21] and heteronuclear[22,23] coupling constant values relative to the fragment of interest were calculated for each conformer, after which the Boltzmann averaged values were computed taking into account the weighted contribution of each conformer to the total population. For example, the $^3J_{HH}$ value for H-4/H-5 in the diastereomeric series **X** (3.9 Hz) originates from a 58% contribution of their *gauche*^{P} (+67.4°) arrangement in conformers **Xa** and **Xe** ($^3J_{HHcalc}$= 2.0 Hz), from a 29% relative to their anti (173.8° and 175.4° respectively) arrangement in **Xb** and **Xd** ($^3J_{HHcalc}$= 8.6 Hz), and from a 13% relative to their *gauche*^{M} orientation (−62.7°; $^3J_{HHcalc}$=1.7 Hz) present in conformer **Xc**. A comparison of the Boltzmann averaged calculated *J* values thus obtained with their corresponding experimental counterparts reveals, for the diastereoisomer **X**, a very satisfactory agreement between theoretical and experimental data over the whole pattern of *J*-couplings (Table 4).

Moreover, looking at Table 4, it can be observed that for diastereoisomer **Y** three out of five calculated *J* values are not in accordance with the experimental data; notably, the $^2J_{H5C4}$ differs from the target value by more than 3 Hz.[27]

These results confirmed that the NMR data of sapinofuranone A can be fully accounted for by a multiple conformational equilibrium of diastereoisomer X (4*R**,5*S**) with its rotamers interconverting in a fast regime. In this view, the observation of averaged *J*-coupling values can be explained in terms of a significant participation of all the staggered rotamers to the total population.

In conclusion, the encouraging results presented in this paper are just one initial demonstration of the interesting g potential applications that may be devised by careful use of computational techniques to complement advanced spectroscopic techniques, such as multidimensional NMR, in the determination of the relative stereochemistry of organic molecules. An extension of the methodology discussed here to other selected systems with known (or independently determined) configuration will be reported in due course.

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[12] It is important to point out that for the dioxygenated C4−C5 fragment, $^2J_{CH}$ values are expected in the range +3 to −5 Hz. Therefore a $^2J_{CH}$ of about −3 Hz, which in other systems may be regarded as a medium/small coupling, in this particular context has to be considered as a medium/large *J* value.

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[20] The absolute HF energies provided for each conformer by ab initio calculations were used to calculate Boltzmann weighting factors at 298.15 K. Test calculations showed that the contribution of entropic terms (estimated by computing molecular frequencies) to Gibbs free energies may be neglected without affecting the final results to any significant degree.

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[24] The calculation of the vicinal coupling constants $^3J_{HH}$ from dihedral angles, taking into account the electronegativity of the substituents of the stereocenters, was done with use of the Altona equation.[21]

[25] Theoretical curves, obtained by carring out ab initio calculations on fragments bearing electronegative substituents, and reporting the angular dependence of the coupling constant vs the dihedral angle, have been used for the extrapolation of the $^{2,3}J_{CH}$ values.[22, 23]

[26] The average *J* values were calculated in accordance with the Boltzman distribution by using the following equation: $J = (\sum^{N_i=1}[J_{xi}\, e^{-\Delta G°_i/RT}])/(\sum^{Ni>1}[e^{-\Delta G°_i/RT}])$, where *N* is the number of conformers found for each isomer (X and Y), *R* is the ideal gas constant, *T* is the absolute temperature, and $\Delta G°_i$ is the standard free energy value of the *i*th conformer relative to the energy of the most stable conformer. The J_{xi} values, their average *J* values, and experimental *J* are reported in Tables 2 and 3.

[27] Unfortunately, the limited amount of sapinofuranone B currently available (0.5 mg) did not allow us to obtain the full set of heteronuclear *J* couplings for this compound also. This prevented us from comparing its experimental data with the theoretical *J* values derived for the diastereomer Y.