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Stereochemical analysis of natural products. Approaches relying on the combination of NMR spectroscopy and computational methods*,†

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Abstract: The stereochemical study of flexible stereogenic carbon chains, such as those of many novel natural products, is a particularly challenging task. Recent applications of our group on the so-called "*J*-based approach", a methodology relying on a detailed analysis of homonuclear (H–H) and heteronuclear (C–H) $^{2,3}J$ couplings, include the study of the sphinxolide family of antitumor macrolides, a group of molecules characterized by a flexible macrocyclic framework bearing a number of oxygenated and methylated undetermined stereocenters, and of ascaulitoxin, a nitrogen-containing phytotoxin with herbicidal activity produced by a phytopathogenic fungus. An extension of the original procedure, relying on a Hartree–Fock (HF) ab initio calculation of conformational equilibrium and an estimate of the Boltzmann averaged $^{2,3}J_{HH}$ and $^{2,3}J_{CH}$ couplings, has been applied to the stereochemical study of sapinofuranone A, where the conformational equilibrium among existing rotamers had initially led to controversial results. ^{13}C NMR chemical shifts are additional useful parameters in the study of complex organic molecules. Along these lines, we have lately proposed the use of Hartree–Fock gauge including atomic orbitals (GIAO) calculated ^{13}C NMR chemical shift values as a supporting tool for the validation of the structure of new natural products and the determination of the relative stereochemistry of diastereomeric flexible compounds that are characterized by multiple conformer equilibria.

INTRODUCTION

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. NMR spectroscopy is certainly one of the most powerful instrumental techniques for the analysis of the relative configuration of organic compounds. Even more, the application of NMR techniques to the problem of determining the relative spatial orientation of substituents has become, in selected classes of asymmetric compounds, a routine task in modern organic chemistry laboratories. The stereochemical analysis of compounds with well-defined conformational properties is presently fairly easy to accomplish, given the wealth of high-resolution NMR experiments useful in these kinds of studies. Typically, cyclic compounds with small (three- to six-membered) rings display a predictable conformational behavior allowing the knowledge of their configuration to be extracted from simple NMR parameters, such as proton–proton *J*-coupling values and/or nuclear Overhauser effect (NOE) intensities. A much more challenging task is the assignment of

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†Dedicated to the memory of Prof. Paul J. Scheuer, beloved mentor in science and great friend in life. R.R.

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relative (and absolute) configuration in the case of flexible systems, such as open polysubstituted chains and macrocyclic compounds for which a clear-cut, definite strategy of stereochemical analysis is not yet available. Traditionally, these kinds of difficulties have been expressed in a sort of NMR stereochemical paradigm: if you already know the conformation of a molecule you may easily get its configuration; conversely, from the configuration of a system one may derive its conformation (as in the case of NMR studies of biopolymers). This has to do with the degree of geometrical uncertainty that one can simultaneously deal with by NMR on complex molecules.

Recently, we have become interested in probing various techniques in the attempt to overcome these limits in the NMR study of stereochemically undetermined flexible systems, notably of complex natural products. From a methodological viewpoint, these studies can be conveniently divided into approaches relying on the extensive use of *J* values and approaches exclusively based on chemical shift arguments.

J-BASED APPROACHES

General considerations

The well-known phenomenon of scalar coupling supplies important NMR parameters, the coupling constant (*J*) values, which have proven extremely useful for conformational and stereochemical studies of organic molecules, providing relevant geometric information. In particular, the coupling constants between protons separated by three bonds ($^3J_{HH}$) are directly related to their dihedral angles through the famous Karplus equation [1]. More recently, it has been shown that proton–proton vicinal coupling constants depend from a number of other molecular parameters, such as substitution, bond angles, bond lengths and more effectively from the electronegativity and the relative position of the substituents bound to the H–C–C–H fragment. Accordingly, a new empirical equation has been formulated which, taking into account the substitution pattern, allows an accurate prediction of *J* values from dihedral angles and vice versa [2].

Likewise, heteronuclear (^{1}H–^{13}C) vicinal coupling constants ($^3J_{CH}$) follow a Karplus-like relationship and, therefore, can be used to derive additional angular constraints. The $^2J_{CH}$ values, which involve nuclei not describing a dihedral angle, can still be useful when the α-carbon bears an electronegative substituent, like an oxygen or a halogen. In this case, the relative magnitude of the two-bond coupling constant can be related to the dihedral angle between the proton and electronegative atom bound to the coupled carbon (Table 1) [3].

Original *J*-based methodology

On the basis of the above observations, an NMR *J*-based configuration analysis for the stereochemical determination of acyclic structures has recently been devised by Murata and coworkers [4] and has been applied to the study of several natural products [5–8]. This method, based on the combined use of homonuclear proton–proton coupling constants $^3J_{HH}$, heteronuclear proton–carbon coupling constants $^{2,3}J_{CH}$ and NOE data, is particularly suitable to acyclic structures having stereogenic carbons bearing hydroxy, alkoxy, or methyl substituents.

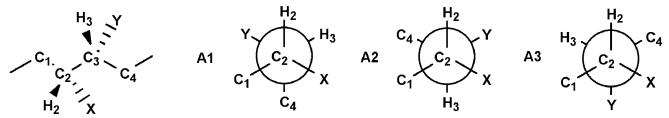
In acyclic systems all coupling constants are observed as a weighted average of those due to each conformer, and for this reason, the dihedral angles obtained from these data provide a much more reliable constraint information regarding the major conformer than NOE data. However, the use of the sole homonuclear coupling constants ($^3J_{HH}$) for relative stereochemistry assignments in flexible systems is not feasible because they do not allow to distinguish between all the spatial arrangements of the substituents linked to a two-carbon fragment, as it would be needed for the identification of the rotamer(s) with correct configuration. In other words, even if we restrict our attention just to staggered conformations, in absence of additional information on the geometry of the system, one angular constraint for

each C_2 segment is not sufficient to solve the problem, because more solutions are consistent with the experimental data. The situation changes dramatically, though, when additional angular information on a stereochemically undetermined C_2 fragment is provided from heteronuclear coupling constants ($^{2,3}J_{CH}$). The latter *J* values, although widely used in NMR studies of labeled biopolymers [9], have been rather inaccessible to organic chemists for quite a long time. Nowadays, thanks to the progress in 2D NMR techniques, hardware improvement and the diffusion of high-field magnets (500–600 MHz), these *J* couplings are measurable on compounds at ^{13}C natural abundance.

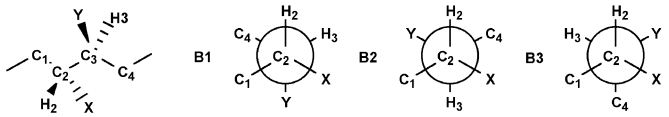
Every chiral molecule containing consecutive or alternate stereochemical centers can be ideally divided in two-carbon fragments. This simplification allows us to determine, for each single fragment, the predominant rotamer with correct configuration, among the six possible staggered conformers through the use of the *J*-based NMR approach (Scheme 1).

For the sake of simplicity, let us compare every single couple of vicinal asymmetric carbons to those belonging to a 2,3-disubstituted butane system. The strategy followed for a 2,3-disubstituted butane can then be applied to a large variety of natural and synthetic products in which the substituents are methoxy, hydroxy, and methyl groups.

A 2,3-disubstituted butane can have two relative diastereomeric configurations, named *syn* (or *threo*) and *anti* (or *erythro*). Each of these two configurations can be arranged in three staggered rotamers, for a total of six possible conformers, as shown in Scheme 1 (A1–A3, B1–B3).



***threo* (*syn*)**				
	$^3J_{H2H3}$	small	large	small
	$^3J_{H2C4}$	large	small	small
	$^3J_{H3C1}$	large	small	small
	X = Me, Y = OR			
	$^3J_{CxH3}$	small	small	large
	$^2J_{C3H2}$	large	large	small
	X = OR, Y = OR			
	$^2J_{C2H3}$	large	large	small
	$^2J_{C3H2}$	large	large	small



***erythro* (*anti*)**				
	$^3J_{H2H3}$	small	large	small
	$^3J_{H2C4}$	small	small	large
	$^3J_{H3C1}$	large	small	small
	X = Me, Y = OR			
	$^3J_{CxH3}$	small	small	large
	$^2J_{C3H2}$	small	large	large
	X = OR, Y = OR			
	$^2J_{C2H3}$	large	large	small
	$^2J_{C3H2}$	small	large	large

Scheme 1 Identification of the single conformer with correct configuration from the staggered rotamers with *threo* (*syn*) and *erythro* (*anti*) arrangements through the combined use of measured $^3J_{HH}$ and $^{2,3}J_{CH}$ values.

Table 1 $^{3}J_{HH}$ and $^{2,3}J_{CH}$ (Hz) coupling constant values for *anti* and *gauche* arrangements in acyclic systems.

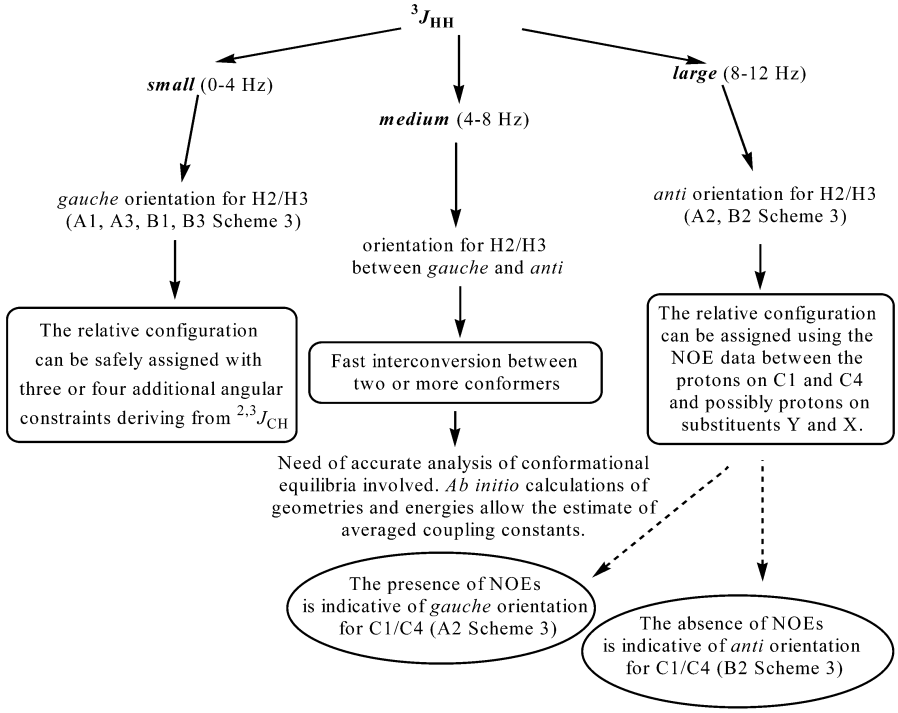
	Anti			***Gauche***		
Oxygenated functionalities	$^{3}J_{HH}$ (*large*)	$^{3}J_{CH}$ (*large*)	$^{2}J_{CH}$ (*small*)[a]	$^{3}J_{HH}$ (*small*)	$^{3}J_{CH}$ (*small*)	$^{2}J_{CH}$ (*large*)[b]
None	9–12 Hz	6–8 Hz	–	2–4 Hz	1–3 Hz	–
One	8–11 Hz	6–8 Hz	0 to –2[c] Hz	1–4 Hz	1–3 Hz	–5 to –7[c] Hz
Two	7–10 Hz	5–7 Hz	2–0[d] Hz	0–3 Hz	1–3 Hz	–4 to –6[d] Hz

[a,b]Oxygen functions on relevant carbons are *gauche* and *anti* to their vicinal protons.
[c2]J_{CH} values are those between an oxygenated carbon and a proton on the neighboring carbon.
[d2]J_{CH} values are those between an oxygenated carbon and a proton on the neighboring oxygenated carbon.

Every single rotamer shows its own homonuclear and heteronuclear coupling constant pattern.

Assuming that we have a sufficient number of experimental coupling constraints, these $^{3}J_{HH}$ and $^{2,3}J_{CH}$ will allow us to univocally identify four of the six rotamers, namely A1, A3, B1, B3. The two rotamers A2 and B2, characterized by an *anti* position of the two vicinal protons, have to be distinguished on the basis of additional NOEs (or rotational nuclear Overhauser effects, ROEs) data because they show the same coupling constant pattern.

The first step in the determination of the relative configuration of two vicinal methines consists of the evaluation of the homonuclear proton–proton coupling constant of the C_2 fragment of interest (Scheme 2), in order to assess whether or not it exists in a predominant conformation.

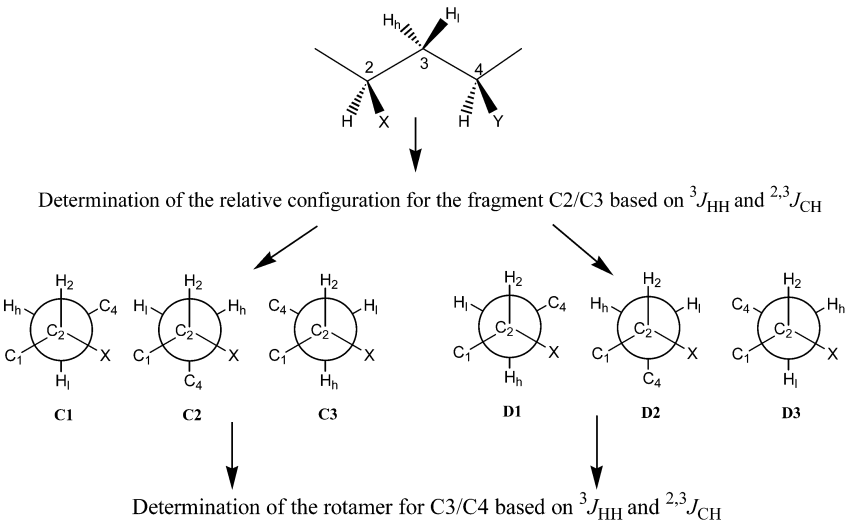


Scheme 2 Some considerations on the applicability of the *J*-based methodology.

Small $^{3}J_{HH}$ (0–4 Hz) will indicate a *gauche* arrangement of H2 and H3 protons. In this case, the relative configuration can be safely obtained from three or four additional angular constraints deriving

from the heteronuclear coupling constants values. These $^{2,3}J_{CH}$ values (Table 1), indeed, will allow us to establish the arrangement (*gauche* or *anti*) of H2 with regard to substituents Y and C4, and the arrangement of H3 with regard to substituents X and C1 and will therefore allow us to choose the correct *gauche* rotamer among A1, A3 (*threo*), B1 and B3 (*erythro*) (Schemes 1 and 2). Large $^{3}J_{HH}$ values (8–12 Hz) indicate *anti* disposition of the protons. Nevertheless, the $^{2,3}J_{CH}$ values are the same for both rotamers A2 (*threo*) and B2 (*erythro*) (Schemes 1 and 2), and so they don't allow us to distinguish them. In this case, it is necessary to use NOE data regarding protons on carbons C1 and C4 and possibly protons on substituents Y and X. An NOE effect between protons on C1 and C4 and/or an NOE effect between protons on Y and X suggest the rotamer A2 in Scheme 1, while an NOE between protons on C1 and on Y and/or an NOE between protons on C4 and on X are suggestive of the rotamer B2. Intermediate values of $^{3}J_{HH}$ (4–8 Hz) are usually indicative of an interconversion between two or more conformers that is fast on the NMR scale. In this case, it would be still possible to identify the alternating conformers on the basis of the homonuclear and heteronuclear coupling constant values and to assign the relative configuration, but the methodology is being pushed to the limit, and, therefore, much more care has to be put into its application. In addition, the registration of NMR spectra at a low temperature can be of help in the case of complex conformational equilibria, owing to the population increase of the most stable conformer.

Often organic molecules contain, together with a number of adjacent stereocenters, asymmetric carbons separated by one methylene. The *J*-based configurational analysis has been shown to be useful in assigning the relative configuration of this type of system (Scheme 3). This is possible because, generally, the two diasterotopic methylene protons can be stereospecifically assigned, and then the methylene carbon can be treated in the same way as if it were an asymmetric center with adjacent methines, as we have seen before. Clearly, two consecutive assignments will be necessary in order to get the relative spatial arrangement of substituents lying on carbons separated by a methylene.



Scheme 3 Strategy to assign the configuration of the two methines separated from a methylene.

For instance, let us examine a three-carbon system (Scheme 3), in which two carbons are asymmetric methines (C2 and C4) and the central one is a methylene (C3). The two protons bound to C3 can be distinguished on the basis of their chemical shift (H_h and H_l are the high- and low-field protons, respectively). The first step consists of the determination of the predominant rotamer for the fragment C2–C3, in analogy with the case of two adjacent methines. Also, in this case the possible rotamers can

be univocally distinguished on the bases of $^{3}J_{HH}$ and $^{2,3}J_{CH}$ and further supported through the analysis of the NOE data.

After assessing the relative configuration of the fragment C2–C3, we can establish the diastereomeric relationship between the same methylene C3 and the second (C4) methine as well, leading us to extend the diastereomeric relationship between the two methines through the stereospecific assignment of the methylene protons. It is interesting to notice that, in case of spectra with very good chemical shift dispersion, this method is in principle also applicable to systems of asymmetric methines separated by an ethylene group, even though in these cases the real problem is the conformational averaging usually associated to unsubstituted chains.

In conclusion, the method introduced by Murata allows us to determine the relative configuration of systems with vicinal asymmetric methine groups and/or separated from one (or more) methylene group(s), provided that a sufficient number of accurately measured *J*-coupling values is available.

For small molecules, the homonuclear $^{3}J_{HH}$ can be easily determined through the analysis of a 1D proton spectrum. Nevertheless, the use of more sophisticated 2D NMR techniques, such as exclusive correlation spectroscopy (E.COSY) [10] or primitive exclusive correlation spectroscopy (P.E.COSY) [11], is mandatory when the direct measurement of the *J* values in the proton spectra of medium- and high-molecular-weight products is prevented by severe signal overcrowding.

Experimental measurements of $^{2,3}J_{CH}$ couplings

Heteronuclear long-range $^{2,3}J_{CH}$ values have become widely available after the introduction of inverse detection NMR techniques [12] and the implementation of pulse-field-gradient (PFG) hardware in commercial NMR spectrometers. These values are determined, in the original method proposed by Murata, by 2D hetero half-filtered TOCSY (HETLOC) [13,14], and phase-sensitive heteronuclear multibond correlation (PS-HMBC) [15–18] experiments, and, in a recent application of the methodology, heteronuclear single quantum correlation (HSQC)-TOCSY spectra have also been used for this purpose [19].

HETLOC is a two-dimensional homonuclear correlation experiment in which the conventional TOCSY-type peaks are complicated in both dimensions by heteronuclear couplings. In particular, a peak in the spectrum corresponding to a long-range coupling between two protons ($\omega_2 = H_x$ and $\omega_1 = H_y$) (Fig. 1) will show a large ω_1 signal displacement due to the heteronuclear direct coupling between H_y and C_y ($^{1}J_{CyHy}$), which is not useful for our purposes but which allows us to measure efficiently the small ω_2 peak splitting due to the heteronuclear long-range coupling between proton H_x and C_y ($^{2}J_{CyHx}$ or $^{3}J_{CyHx}$). The limitation in the HETLOC method lies in the intrinsic nature of the peaks: the two protons must belong to the same spin system and must exhibit a TOCSY correlation peak. For this reason, it is impossible to use this experiment to measure coupling constants between a proton and a quaternary carbon or a proton and a carbon belonging to a different spin system.

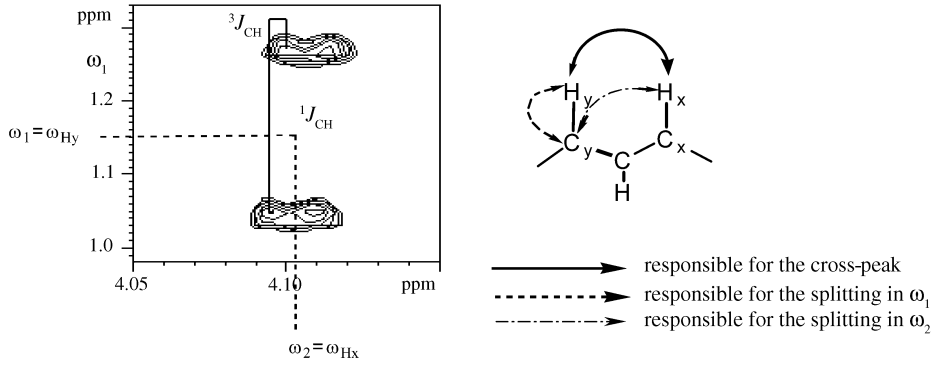


Fig. 1 Direct measurement of $^{2,3}J_{CH}$ coupling constants through analysis of HETLOC cross-peaks.

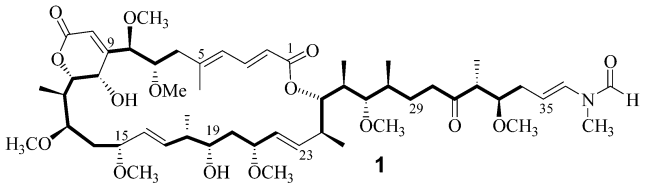
Also, $^{2,3}J_{CH}$ measurements for natural products can be biased from a low sensitivity owing to the natural abundance of 1 % of the ^{13}C. A PFG-enhanced version of the HETLOC experiment has been reported [20]; this overcomes the problem of the low sensitivity, while it is not efficient in the elimination of the severe signal overlap frequently seen in the spectra of natural products. The resonance overlap in the HETLOC spectra can be often overcome through a PFG version of the 2D-HSQC-TOCSY experiment, recently proposed for measuring heteronuclear long-range coupling constants [21] and applied in the context of a stereochemical study of the marine natural product sphinxolide [19] (see below). In the HSQC-TOCSY, the proton–proton connectivities are filtered by the ^{13}C chemical shift relative to the carbon connected to the proton from which the magnetization is generated. The information relative to the heteronuclear coupling constants can be obtained using a ^{13}C-decoupled and a ^{13}C-coupled HSQC-TOCSY spectrum followed by a computer-aided comparison of the in-phase ^{1}H multiplet with and without the heteronuclear splitting. As for the HETLOC experiment, the main limitation of this technique is due to the fact that only heteronuclear long-range couplings between protons and protonated carbons belonging to the same spin system are measurable, because also in this case the magnetization is transferred via a homonuclear Hartman–Hahn effect. The HMBC experiment, usually performed for the structural characterization of natural and synthetic organic molecules, is a heteronuclear correlation technique that provides information on long-range couplings of proton and carbon separated by two or three covalent bonds. In the PS-HMBC experiment, it is possible to quantitatively analyze the proton-carbon correlations, and, from their relative intensity, it is possible to extrapolate the $^{2,3}J_{CH}$ values (eq. 1):

$$I_{CaH}/I_{CbH} = \sin^2 (\pi^{2,3}J_{CaH}\,\Delta)/\sin^2 (\pi^{2,3}J_{CbH}\,\Delta) \quad (1)$$

where I_{CaH} and I_{CbH} are the volumes of the cross-peaks due to C_a-H and C_b-H couplings, respectively, while Δ is the delay of long-range proton-carbon coupling evolution, usually set at 50 ms (corresponding to a maximum J value of 10 Hz). So, a given heteronuclear J value, for instance, $^{2,3}J_{CaH}$, can be obtained by using the experimentally determined cross-peak volumes I_{CaH} and I_{CbH} and by the value of $^{2,3}J_{CbH}$ that needs to be independently measured, for instance, by HETLOC spectra. This technique is much more sensitive than the HETLOC (especially in its gradient-enhanced version PFG-PS-HMBC [22]), and it offers a better spectral dispersion through the ω_1 carbon dimension. Moreover, the correlations measured in the PS-HMBC are not read through homonuclear TOCSY correlations, like in the HETLOC spectrum, and for this reason it is possible to get coupling constants between protons and quaternary carbons or protons and carbons belonging to different spin systems.

Application of the *J*-based methodology to the sphinxolide system

Sphinxolide (**1**), the parent member of a family of cytotoxins derived from marine sponges of the order Lithistida, is a potent antitumor marine 26-membered macrolide capable of specifically targeting cell microfilaments, causing their disruption at nanomolar concentration, and exhibiting ability to circumvent multidrug resistance (MDR) in cancer cells [23]. The sphinxolide molecule, featuring 17 stereogenic centers in a rather flexible carbon framework, appeared, with its side chain and large macrolactone ring, a particularly interesting candidate for the application of a *J*-based methodology [19]. The possibility to extract heteronuclear *J*-couplings from a variety of different 2D spectra was very instru-



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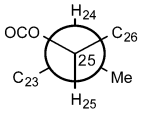
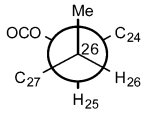
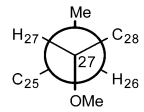
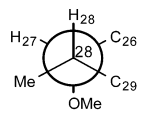
mental to the stereochemical study of this system. Indeed, 12 $^{2,3}J_{CH}$ values that could not be measured by means of HETLOC spectra were obtained from the comparison of suitable ω_2-slices of coupled and uncoupled HSQC-TOCSY spectra. Table 2 reports the dominant rotamers for the fragment C24–C28 of **1** along with the relevant NMR data used to derive the found relative configurations.

Table 2 Dominant rotamers for the fragment C24–C28 of sphinxolide (**1**) along with their relative configurations (T = 300 K, in $CDCl_3$). ROESY contacts are classified into strong (s), medium (m), and weak (w) effects.

Fragment	Segment	$^3J_{HH}$ (Hz)	Selected $^{2,3}J_{CH}$ (Hz) [a]	Selected ROEs	Total number of $^{2,3}J_{CH}$
C24–C25 *anti*		10.0	$^2J_{H24C25}$ –6.7 (HT and HL) $^3J_{H24C26}$ 1.5 (HT and HL) $^3J_{H25Me24}$ 1.4 (HT and HL) $^3J_{H25C=23}$ 2.8 (HT and HL)	H26-Me(s)	4 + 4[b]
C25–C26 *syn*		1.3 2.6[b]	$^2J_{H26C25}$ –0.8 (HT and HL) $^2J_{H26C25}$ –0.9[b] (HB) $^3J_{H25Me}$ 5.0[b] (HB) $^3J_{H25C27}$ 2.9[b] (HB)	H25-H27(m) H24-Me(s) H26-H24(m)	4 + 4[b]
C26–C27 *anti*		9.8	$^2J_{H26C27}$ –6.6 (HT and HL) $^3J_{H27Me26}$ 1.8 (HT and HL) $^3J_{H26C28}$ 1.5 (HT and HL)	H28-Me(m) H25-OMe27(m)	4 + 4[b]
C27–C28 *anti*		2.4	$^2J_{H28C27}$ –1.9 (HB) $^3J_{H27Me28}$ 2.8 (HT only)	H27-Me(s) H26-H29b(m)	4 + 3[b]

[a]The following short-hand notations have been used: HT = PFG-HSQC-TOCSY, HB = PS-PFG-HMBC, and HL = PFG-HETLOC.

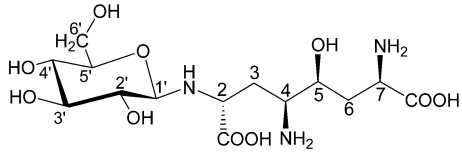
[b]*J*-values and ROEs measured at 276 K.

From a methodological viewpoint, the analysis of the C13–C15 segment can be instructive. The stereochemical assignment of this and other regions of the molecule was, in fact, complicated by a rapid interconversion of different conformers, as indicated by the intermediate values of both homo- and heteronuclear coupling constants (see Scheme 2). Inspection of the spectra acquired at 276 K showed that in these conditions the population of the lowest energy conformer was increased up to the point where the methodology could be safely applied. As an example, $^3J_{H14a–H15}$ and $^3J_{H14b–H15}$ changed from the intermediate values of 7.8 and 6.2 Hz to 6.0 and 15.0 Hz, respectively, allowing to unambiguously establish a *gauche* relationship for H14a–H15 and an *anti* relationship for H14b–H15. Similar patterns were observed in the temperature dependence of heteronuclear *J* values relative to the same fragment, allowing full spectroscopic characterization of this subunit. However, one has not to conclude that acquisition of NMR data over a wide range of temperatures provides a final answer for any kind of stereoassignment in presence of multiple-conformer equilibria. For instance, neither the pattern of *J*-couplings nor ROEs at any temperature in the 276–328 K range allowed a safe stereochemical assignment for the stereocenters belonging to the C18–C19 unit. Thus, the determination of the configuration of this portion of the molecule had to be addressed by a chemical approach, involving the degradation of the intact natural product to furnish the segment of interest followed by the stereoselective synthesis of all its possible stereoisomers [24].

Finally, it has to be pointed out that, despite the methodology is usually referred to as *J*-based approach, dipolar contacts, often obtained from rotational nuclear Overhauser effect spectroscopy (ROESY) [25] data, also play an important role for such stereochemical assignments. In this respect, we found that ROEs could be utilized in this study at two different levels. In fact, proximity contacts between protons within a given C_2 fragment are of crucial importance when a proton–proton *anti* arrangement is present and can generally serve to increase the reliability of relative configuration assignments. Moreover, we found that dipolar couplings were also useful in the analysis of spatial relationships of longer carbon sequences and in many cases provided an independent and more global level of assessment of the 3D features of the system, allowing a cross-check of the configurations already assigned to the individual C_2-units.

Ascaulitoxin molecule: A stereochemical study of a nitrogen-containing system

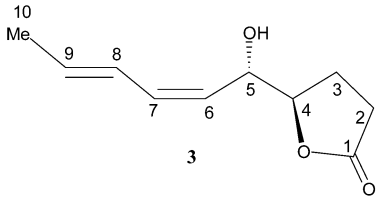
As pointed out above, the *J*-based method has proved particularly useful for the configurational analysis of polyoxygenated/polymethylated frameworks, typically found in polyketides of natural origin. So far, the method (relying on $^2J_{CH}$ couplings) has not been extended to the stereochemical analysis of nitrogen-substituted chains, such as the ascaulitoxin molecule (**2**). However, we envisaged that this approach was likely to display more breadth of application, since the need of oxygen substituents (hydroxy, methoxy, etc.) stemmed just on the need of suitable correlations between the values of $^2J_{CH}$ couplings and the dihedral angles between the proton and the heteroatom attached to the carbon coupled to the proton. Indeed, in oxygenated (and also dioxygenated) C_2-fragments, small $^2J_{CH}$ couplings (in absolute value, e.g., +2 → –1 Hz) are commonly observed for *anti*-like arrangements between the proton and the oxygen, whereas large (in absolute value, e.g., –4 → –6 Hz) are found for *gauche*-like arrangements. For the case of molecular fragments containing nitrogen substituents, although there is still need of experimental measurements of $^2J_{CH}$ couplings in systems with known or predictable structural features, reliable curves describing their angular dependence have been obtained by ab initio methods. In fact, a recently published review covering the angular dependence of a variety of heteronuclear couplings [26] has drawn our attention on the comparative influence of oxygen and nitrogen substituents on $^2J_{CH}$ couplings and suggested that an extension of the *J*-based approach to nitrogen-containing molecules was indeed possible. In fact, our experimentally derived data on the bis-amino acid ascaulitoxin (**2**) would indicate a good correlation between calculated and measured couplings of the latter type (see Table 1). In this respect, $^2J_{CH}$ couplings at nitrogen-bearing carbons display a pattern of values qualitatively similar (although within a somewhat tighter range) to that of oxygenated fragments, ranging between ca. –1 Hz (*anti*-like arrangements) to ca. –4 Hz (*gauche*-like arrangements).



2

Extension of the *J*-based method to fast multiple-conformer equilibria

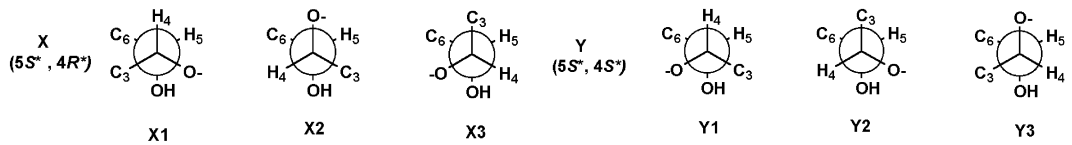
A major limitation of the *J*-based approach in its original formulation is its inability to treat systems exhibiting multiple-conformer equilibria and thus characterized by averaged NMR parameters. In order to overcome this drawback, a new strategy, extending the application of the *J*-based configuration analysis to such systems has been very recently described by our group and applied to sapinofuranone A (**3**) [27], a phytotoxic molecule produced by three strains of *Sphaeropsis sapinea* [28].



3

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 that can be compared against experimental NMR data. In particular, we reasoned that this intrinsic limit of the methodology could have been overcome by using ab initio calculations to predict the molecular geometries and relative energies of all relevant conformers for 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 of all interconverting conformers. Theoretical data of each stereoisomer, such as *J*-couplings, can be carefully examined against the experimental NMR data of the compound with unknown configuration, leading, in case of a satisfactory match for *only one* of the possible diastereomers, to the determination of its relative configuration.

The set of NMR data deriving from the application of the plain *J*-based strategy to **3** could not unambiguously identify only one among the six possible rotamers (Scheme 4) 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. As a matter of fact, if one tries to interpret the *J*-coupling values as arising from a single conformer, the *J*-based analysis would lead to conflicting results. Once we had established that the *J*-based methodology in its original formulation was not suitable for the stereochemical study of **3**, 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 aimed to the identification of the representative conformers for each stereoisomeric series; (b) geometry optimization at the Hartree–Fock (HF) level of the so-obtained conformers followed by the calculation of their heat of formation; (c) determination of the Boltzmann weighting factors of all the representative conformers for each stereoisomer; (d) computation of the averaged *J*-couplings and then comparison between calculated and experimental *J* values ($^{2,3}J_{CH}$, $^{3}J_{HH}$) [29–31].



Scheme 4 Possible staggered rotamers for the two diastereoisomers (X and Y) of sapinofuranone A.

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 3). Moreover, looking at Table 3, 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 $^{2}J_{H5C4}$ differs from the target value by more than 1 Hz.

Table 3 Experimental (T = 298 K, in $CDCl_3$) and calculated *J* values of sapinofuranone A (**1**). Calculated *J* values differing more than 1 Hz with respect to experimental values are displayed in bold.

Experimental *J* values (Hz) of **3**		Calculated *J* values (Hz) of the two diastereoisomers of **3**	
		X	**Y**
$^3J_{H4H5}$	3.1 (small)	3.9	3.3
$^2J_{H5C4}$	–3.3 (medium/large)[a]	–3.4	**–0.4**
$^2J_{H4C5}$	1.0 (small) [28]	0.2	**–0.3**
$^3J_{H5C3}$	2.6 (small)[b]	2.9	2.1
$^3J_{H4C6}$	<1.0 (small) [29]	0.9	**2.4**

[a]PFG-HETLOC
[b]PS-PFG-HMBC

APPROACHES RELYING ON ^{13}C NMR CHEMICAL SHIFT ARGUMENTS

^{13}C NMR spectroscopy, despite its inherent low sensitivity, has always been regarded as a powerful means of investigation for the stereochemical characterization of organic molecules. Recently, the remarkable progress in computational methods has opened new avenues of research in the field of NMR chemical shift prediction, with interesting applications in the full characterization of complex natural products. Quantum chemistry methods have, therefore, emerged as a tool for the investigation both of the constitution, the conformation, and the configuration of organic systems, also in the challenging case of flexible systems, namely in presence of high conformational mobility. In a recent study, we showed [32] that ^{13}C NMR theoretical chemical shifts obtained by ab initio calculations employing either HF or density functional theory (DFT) approaches, display good-to-excellent agreements with experimental data, especially for low-polar compounds whose ^{13}C NMR spectra were typically recorded in deuterated chloroform solution. Indeed, for this class of compounds, we neglected the solvent effects in our calculations, with the heuristic assumption that the solution structure is not, or it is very slightly, perturbed by the presence of the low-polarity solvent. The final findings confirmed such hypothesis. A comparison between calculated and experimental chemical shifts of compounds with known structures [8] shows that the computed chemical shifts are in good to excellent agreement with the experimental data. The very good correlation between the experimental and the calculated ^{13}C values for 14 low-polar compounds possessing different chemical functionalities is represented in Fig. 2.

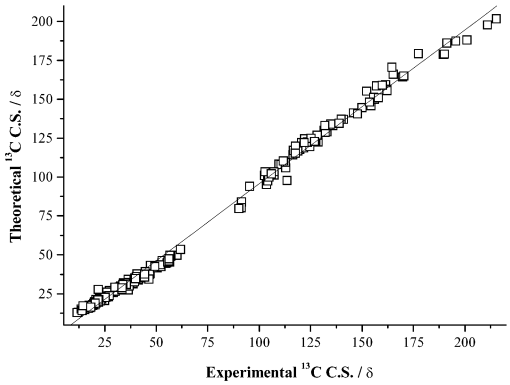
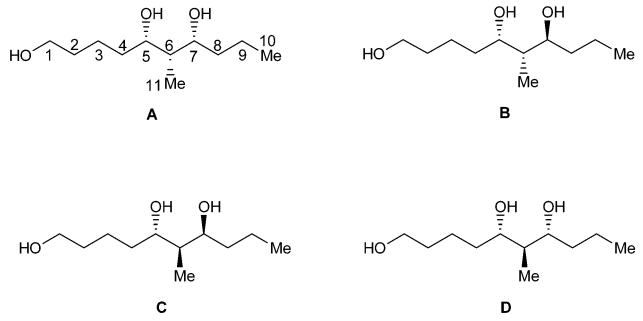


Fig. 2 Correlation plot of calculated vs. experimental ^{13}C NMR C.S., at HF level, for the 14 species examined.

This study showed the capability and the limits of the HF method, using the 6-31g(d) basis set, and the improvements arising at the higher B3LYP level, which performs more on systems where the electronic correlation effects are considerable. The excellent results obtained for this set of compounds can be ascribed to a fundamental factor: the possibility, with the up-to-date availability of low-cost hardware resources, of the ab initio optimization of the geometries of the compound in examination. Indeed, the calculation of any molecular properties strongly depends on the quality of the molecular geometry and hence on the level of optimization of the examined structure. The ^{13}C NMR spectrum interpretation method has been tested on three cases in which the misinterpretation of experimental data led to erroneous structure determinations [33].

Following the encouraging results obtained by calculating the ^{13}C chemical shifts of natural compounds, the method has been applied to the challenging field of the determination of the relative stereochemistry of flexible compounds [34], and it has been tested on the four low-polar diastereomers **A**–**D** depicted in Scheme 5.



Scheme 5 Structure of the four stereoisomers **A**–**D**, whose ^{13}C chemical shifts have been previously reported in literature [35].

In this case, a rigorous conformational analysis needs to be carried out, and the experimental chemical shifts must be compared with the calculated data corresponding to the Boltzmann distribution of all the conformers belonging to the compound in examination. In fact, together with the high-level theory required for the geometry optimization of each conformer, which is necessary for accurate prediction of the chemical shifts, it is also important to obtain accurate differences in energy among the

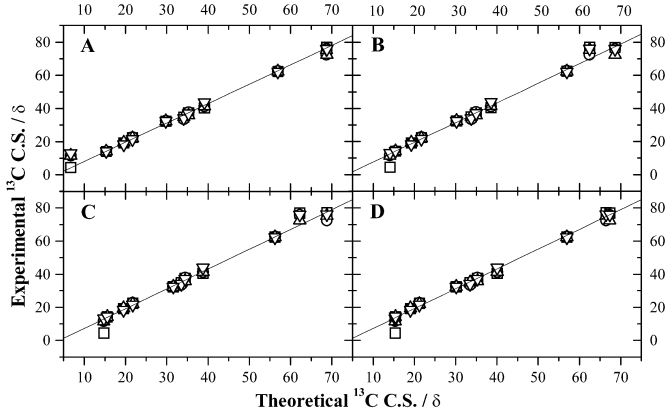


Fig. 3 Correlation plots of experimental vs. theoretical ^{13}C NMR chemical shift values, for the species represented in Scheme 1. In the frames **A**–**D**, the experimental data [33] of the species **A** (□), **B** (○), **C** (△), and **D** (▽) are plotted against the corresponding average theoretical data of **A**–**D**, respectively.

single conformers belonging to each flexible diastereoisomer. This allows us to calculate the average ^{13}C chemical shift values based on a significant Boltzmann distribution of all the conformers. The so-obtained data are compared to the experimental, and the correlation plots are reported in Fig. 3.

It is evident that calculated ^{13}C chemical shifts for **A** and **D** fit the experimental data very well, as reported in Table 4. Nevertheless, stereoisomers **B** and **C** are less unequivocally determined by considering the least-squares linear correlation coefficients. In order to better appreciate the correspondence of the calculated vs. the experimental data, the parameter $\Delta\delta$ (defined as the difference between scaled theoretical and experimental ^{13}C chemical shift values) has been used, and the sum of the $\Delta\delta$ values are reported in Table 5 for all the diastereomers.

Table 4 Correlation coefficients of least-squares linear fits of the theoretical vs. experimental isomer shift correlation plots shown in Fig. 3.

	A calc	**B calc**	**C calc**	**D calc**
A exp	**0.999(4)**	0.992(0)	0.991(5)	0.992(3)
B exp	0.995(5)	**0.998(3)**	0.993(0)	0.998(2)
C exp	0.996(0)	0.994(3)	**0.998(4)**	0.998(0)
D exp	0.994(0)	0.995(9)	0.996(0)	**0.999(3)**

Table 5 Sum of $|\Delta\delta|$ values of theoretical-experimental chemical shifts relative to the four stereoisomers **A**–**D**.

	A calc	**B calc**	**C calc**	**D calc**
A exp	**6.77**	17.95	17.29	19.98
B exp	26.68	**10.91**	15.89	16.08
C exp	26.50	18.47	**9.93**	15.10
D exp	23.42	12.42	12.68	**7.96**

From the examination of Table 5, it is possible to assess that, at this level of theory, the calculated ^{13}C chemical shift of each stereoisomers fit very well the experimental, thus suggesting a computational tool in the determination of the relative stereochemistry of low-polar medium-sized flexible compounds.

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