Volume 953, issues 1–3, 15 August 2010
ISSN 0166-1280
ELSEVIER

JOURNAL OF MOLECULAR STRUCTURE

THEOCHEM

Theory and Computation in Chemistry

CLIFFORD DYKSTRA, AJIT J. THAKKAR and MANUEL YÁÑEZ

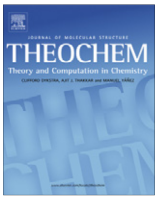
www.elsevier.com/locate/theochem

Journal of Molecular Structure: THEOCHEM 953 (2010) 83–90

Contents lists available at ScienceDirect

Journal of Molecular Structure: THEOCHEM

journal homepage: www.elsevier.com/locate/theochem



Advances in correlation between experimental and DFT/GIAO computed ^{13}C NMR chemical shifts: A theoretical study on pentacyclic terpenoids (fernenes)

Eduardo J. Borkowski [a], Fernando D. Suvire [a], Ricardo D. Enriz [a,b,*]

[a] *Departamento de Química, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco 915, 5700 San Luis, Argentina*
[b] *IMIBIO-CONICET, UNSL, Chacabuco 915, 5700 San Luis, Argentina*

ARTICLE INFO

Article history:
Received 27 April 2010
Accepted 4 May 2010
Available online 7 May 2010

Keywords:
Fernenes
DFT/GIAO calculations
^{13}C chemical shifts
Chemical shits anisotropy asymmetry

ABSTRACT

The ^{13}C chemical shifts of 15 pentacyclic terpenoids (fernenes) are compared to predicted ^{13}C NMR chemical shifts obtained via empirically scaled GIAO shieldings. We report that accurate (rms error approx. 1.5 ppm) predictions of ^{13}C chemical shifts can be achieved for these fernenes through the use of scaled shieldings calculated from GIAO theory with a relatively small basis set and on the basis of geometries obtained from DFT calculations. The best results (considering a reasonable cost-to-benefit ratio) were obtained from B3LYP/6-31G(d)//B3LYP/6-31G(d) computations. The chemical shifts anisotropy asymmetry (η was included in our calculations, enhancing the correlations between calculated and experimental chemical shifts.

Our results indicate that the inclusion of a scaling factor allow to obtain an excellent correlation between δ_{calc} and δ_{exp}. Also, the inclusion of asymmetry improves this correlation. In addition, taking into account the different conformations and the solvent effects a slight enhancement was obtained in this case.

1. Introduction

Triterpenoids are isopentenoids composed of 30-carbon atoms, and they may possess acyclic, mono-, di-, tri-, tetra- or pentacyclic carbon skeletons. Pentacyclic triterpenoids are dominant constituents of this class and have been widely investigated. Modern NMR spectroscopy has proved to be an exceptional tool to elucidate structure and molecular conformation, especially when chemists are dealing with large molecules like pentacyclic terpenoids. In particular, ^{13}C chemical shifts reflect structural features in a highly sensitive manner, so they are indispensable data for the determination of structure, and eventually, conformations and configurations of organic molecules.

Ab initio NMR calculations are now attainable and accurate enough to explore the relationship between chemical shift and molecular structure. The calculated ^{13}C chemical shift, in particular, appears to be accurate enough to aid in experimental peak assignments. Thus, the combination of high-level *ab initio* optimized geometries, theoretically computed NMR chemical shifts, and experimental NMR data affords a structural tool [1–3] that could now be routinely applied for structural elucidations as well as the characterization of newly synthesized compounds [4]. Practical applications so far are more extensive in the areas of carbocations and boron compounds, where high-level *ab initio* methods including electron correlation are necessary to properly describe structure and bonding [5]. However, many other studies have been oriented towards structural determinations of amides and peptides [6]. The conformational problem of rhodopsin chromophore was also evaluated using this approach [7]. These calculations are possible due to the development, implementation and extensive performance test of several practical methods for chemical shift calculations: IGLO [8] (individual gauge localized orbital), LORG [9] (localized orbital origin) and GIAO [10] (gauge independent atomic orbital). The GIAO methods use basis functions that have specific field dependence. In a comparative study of the GIAO and CSGT [11] methods, it was found that shielding tensor components determined using these methods converge to the same value at sufficiently large basis set; however, GIAO shielding tensor components for atoms other than carbon converged faster with respect to basis set size [12]. For this study we choose the GIAO method to perform the calculations.

Unfortunately, applications of theoretically computed ^{13}C chemical shifts to organic structure determinations have not yet become routine, despite the apparent capability to predict shift of ^{13}C nuclei at a sufficient level of accuracy to allow practical applications. The principal reason for such situation may be the level of theory required for these calculations. It has been empha-

---

[*] Corresponding author at: Departamento de Química, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco 915, 5700 San Luis, Argentina. Tel.: +54 2652 424689x157; fax: +54 2652 431301.
E-mail address: denriz@unsl.edu.ar (R.D. Enriz).

0166-1280/$ - see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.theochem.2010.05.007

sized repeatedly that accurate geometries are essential for these studies. In addition, GIAO calculations at high levels of theory, considering the electron correlation, are necessary to properly reproduce the experimental data. However, such approaches can be quite expensive computationally even for organic molecules of modest size. Obviously, the situation is even worse for medium or large-sized molecules. The question which arises is: which is the reasonable level of theory to calculate ^{13}C chemical shifts, keeping the computational requirement available for the capability of a standard laboratory? The calculations and efforts performed in this work have been directed towards finding a small basis set for GIAO calculations along with non-expensive theoretical calculations to achieve maximum practicality.

Experimental NMR spectra in solution are commonly statistical averages affected by dynamic processes such as conformational equilibrium as well as intra and/or intermolecular interactions. In the present study, the influences of conformational interconversions and solvent effects have also been taken into account.

From a theoretical perspective, a particularly important challenge is to understand the dependence of NMR chemical shielding tensors on structural parameters. The NMR technique is based on the sensitivity of magnetic properties, typically isotropic chemical shieldings (σ_{iso}), to the chemical environment of the nuclei. In solution-state techniques, orientational averaging reduces the detailed information in the NMR chemical shielding tensor to one, readily interpretable, σ_{iso} parameter. However, some non-isotropic properties of chemical shielding tensor can be measured with solution-state NMR methods. In principle, theoretical calculations can exploit these non-isotropic properties to transform the orientationally-independent chemical shielding anisotropy (CSA) parameters into a source of structural information.

Empirically, we have chosen to include chemical shifts anisotropy asymmetry, η, to take into account the anisotropy which cannot be eliminated by orientational averaging. Asymmetry is defined [13] as:

$$\eta = \frac{\sigma_{22} - \sigma_{11}}{\sigma_{33} - \sigma_{\text{iso}}}$$

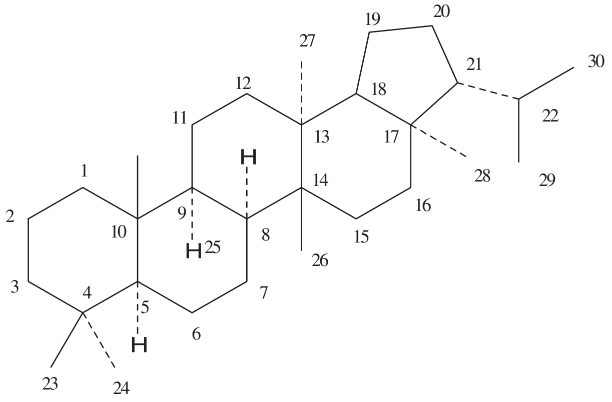
where σ_{ii} are the eigenvalues of the shielding tensor, as calculated by Gaussian 03 [14].

In the present paper, a DFT/GIAO method was used to analyze the experimental chemical shifts of 15 fernenes containing C, H and O. We report that accurate (rms error approx. 1.5 ppm) predictions of ^{13}C chemical shifts can be achieved for these fernenes through the use of scaled shieldings calculated from GIAO theory with a relatively small basis set and on the basis of geometries obtained from DFT (B3LYP/6-31G(d)) calculations. It is interesting to remark that the chemical shifts anisotropy asymmetry (η was included in our calculations, enhancing the correlations between calculated and experimental chemical shifts.

2. Computational details

A set of 15 molecules providing 450 different ^{13}C chemical shifts was employed as a testing set in the present work (Fig. 1); all the experimental data were obtained from Refs. [15,16]. Both, geometry optimization and GIAO calculations were performed with Gaussian 03.

In order to obtain accurate results for a correlated system, it is necessary to introduce the post-Hartree–Fock (HF) methods such as the Moller–Plesset (MP$_n$) perturbation methods [14], the coupled cluster methods, [14,17] the multiconfiguration self consistent-filed methods [18], and so forth. However, calculations with such methods are too expensive to be routinely applied to the chemically interesting large or medium-sized molecules. Density Function Theory (DFT) offers an alternative way to take electron correlation into account, with a reasonable accuracy and a favourable cost-to-benefit ratio. In their pioneering work focused on the comparison of different models for calculating NMR chemical shifts, Cheeseman et al. [12] recommended the B3LYP/6-311+G(2d,p) level of theory for ^{13}C chemical shift predictions. A further systematic study of the ^{13}C performance of GIAO B3LYP76-311+G(2d,p) was reported by Zhang et al. using a set of 18 molecules with various functional groups [19]. There are also large numbers of other reports dedicated to the detailed comparison of 10 or more different approaches in order to achieve a better performance of computational techniques [20–23] as well as hybrid generalized-gradient approximation density functionals [24–26]. It should be noted, however, that we are particularly interested in obtaining a relatively simple method of calculation which can be routinely applied for medium-sized structural elucidations like the fernenes reported here. Thus, in a first step of this work, a preliminary and exploratory analysis was performed by using relatively low-level theory computations. Thus, absolute shieldings were calculated for the compounds in Table 1 via the GIAO method at the B3LYP/6-31G(d,p), B3LYP/6-31G(d), HF/6-31G(d,p) HF/6-31G(d) and HF/3-21G; using the following geometries: B3LYP/6-31G(d), HF/6-31G(d); HF/3-21G and MMFF. A significant number of different possible combinations of GIAO computations and geometries was tested, obtaining the best correlation when using B3LYP/6-31G(d)//B3LYP/6-31G(d) calculations. The GIAO calculations that did not include the effects of electron correlation were the least successful. As an example, for the least energy conformer of compound **1**, the 3-21G/MMFF correlation of experimental shifts with shieldings had an rms error of 7.05 ppm, and 12 (or about one-third) of the calculated shifts differed 5.0 ppm or more from



1: Δ7
2: 28-COOME, Δ7
3: Δ8
4:3β-OH, Δ8
5: 28-COOMe, Δ8
6: 3β, 7α-OH, 11-oxo-Δ8
7: 3β, 11β -OH, 7-oxo-Δ8
8: 3β, 7, 11-dioxo-Δ8
9: Δ9(11)
10: 25-COOH, Δ9(11)
11: 28-COOMe, Δ9(11)
12: 3β, 12α-OH, Δ9(11)
13 (rubiatriol): 3β, 7α, 19α-OH, Δ9(11)
14:2α, 3β-OH, 23-COOMe, Δ9(11)
15: 3β-OH, Δ7

Fig. 1. Structural features of fernenes reported here.

Table 1
Statistical descriptors obtained for the correlation δ_{exp} vs. δ_{calc} in different conditions. The results obtained from Eq. (3) are denoted in bold.

	r^2	Typical error	rms	MAE	Slope (a) ± error	Intercept (b) ± error
Vacuum						
Values corresponding to the least energy conformer						
$\delta 1$	0.9979	1.67	2.62	2.07	$1.044 \pm 2.3 \times 10^{-3}$	-3.30 ± 0.13
$\delta 2$	0.9979	1.67	1.66	1.25	$1.000 \pm 2.2 \times 10^{-3}$	$-2.4 \times 10^{-4} \pm 0.13$
$\delta 3$	**0.9982**	**1.55**	**1.54**	**1.12**	$\mathbf{1.000 \pm 2.0 \times 10^{-3}}$	$\mathbf{-2.0 \times 10^{-4} \pm 0.12}$
Values weighted with Boltzmann proportion						
$\delta 1$	0.99796	1.64	2.61	2.07	$1.044 \pm 2.3 \times 10^{-3}$	-3.31 ± 0.13
$\delta 2$	0.99796	1.64	1.64	1.22	$0.999 \pm 2.2 \times 10^{-3}$	$3.4 \times 10^{-4} \pm 0.12$
$\delta 3$	**0.99824**	**1.53**	**1.52**	**1.10**	$\mathbf{1.000 \pm 2.0 \times 10^{-3}}$	$\mathbf{-5.0 \times 10^{-5} \pm 0.12}$
Solvent						
Values corresponding to the least energy conformer						
$\delta 1$	0.99798	1.64	2.57	2.07	$1.040 \pm 2.3 \times 10^{-3}$	-3.21 ± 0.13
$\delta 2$	0.99798	1.64	1.63	1.22	$1.000 \pm 2.2 \times 10^{-3}$	$5.4 \times 10^{-4} \pm 0.12$
$\delta 3$	**0.99827**	**1.51**	**1.51**	**1.09**	$\mathbf{1.000 \pm 2.0 \times 10^{-3}}$	$\mathbf{2.2 \times 10^{-4} \pm 0.12}$
Values weighted with Boltzmann proportion						
$\delta 1$	0.99800	1.63	2.58	2.07	$1.041 \pm 2.3 \times 10^{-3}$	-3.24 ± 0.13
$\delta 2$	0.99800	1.63	1.62	1.20	$1.000 \pm 2.2 \times 10^{-3}$	$-4.0 \times 10^{-4} \pm 0.12$
$\delta 3$	**0.99828**	**1.51**	**1.50**	**1.08**	$\mathbf{1.000 \pm 2.0 \times 10^{-3}}$	$\mathbf{-3.0 \times 10^{-4} \pm 0.11}$

experimental shifts. Geometry optimizations even at B3LYP/6-31G(d) level are quite computationally expensive. Molecular mechanics calculations are enormously faster than *ab initio* or DFT calculations for geometry optimizations. Thus, in a preliminary survey, the combination of GIAO calculations at B3LYP/6-31G(d) level was examined using MMFF optimizations. The rms obtained for the least energy conformer of compound **1** was 2.36 ppm. Although the correlations obtained using this approach are significant, the obtained rms appears to be too large for predictive applications.

The increase in the used base did not necessarily imply an improvement in the obtained correlation. As an example, the rms obtained using Eq. (1) (see Section 3) for the least energy conformer of **15** is 2.39 ppm using B3LYP/6-31G(d)//B3LYP/6-31G(d), and 2.88 when using B3LYP/6-31G(d,p)//B3LYP/6-31G(d). In turn, the rms obtained using B3LYP/6-311+G(2d,p)//B3LYP/6-31G(d) was 5.53 ppm.

In order to obtain a routine application, it is desirable to use as small a basis set as possible for the GIAO calculations. Our preliminary and exploratory analyses indicate that B3LYP/6-31G(d) GIAO calculations using B3LYP/6-31G(d) geometries gives one of the best cost-benefit ratio. Thus, in this work the commonly used hybrid functional B3LYP [27] was employed, which is based on Becke's three-parameter scheme, consisting of the Slater exchange [28] the exchange functional of Becke 88 [29], and the HF exchange, as well as a mixture of the correlation functional of Vosco–Wilk–Nusair [30] and Lee–Yang–Parr [31]. In all NMR calculations, the GIAO method was employed to circumvent the gauge problem. On the basis of our preliminary results, the 6-31G(d) basis set was adopted. DFT calculations were carried out using Gaussian 03 [14].

Table 2
Parameters obtained for Eqs.)(1)–(3) in different conditions (vacuo or solvent and considering only the global minimum or different conformers).

	σ_{TMS}	m (Eq. (2))	i (Eq. (2))	M (Eq. (3))	I (Eq. (3))
Values corresponding to the least energy conformer					
Vacuum	189.738	−1.0445	194.887	−1.0461	196.000
Solvent	189.820	−1.0403	194.267	−1.0412	195.360
Values weighted with Boltzmann proportion					
Vacuum	189.738	−1.0449	194.940	−1.0463	196.045
Solvent	189.820	−1.0410	194.369	−1.0423	195.444

3. Results and discussion

The general results obtained in this study are summarized in Table 2. Three principal factors affecting the calculations were considered in our computations: (a) the scaling factors, specifically considering the asymmetry, (b) the presence of different conformers, and c) the solvent effects.

3.1. Scaling of GIAO absolute shielding

Previous experimental and theoretical studies of medium and large-sized molecules (peptides and proteins) have indicated some anisotropy dependence on the local chemical surroundings. However, structural variations of CSA parameters in medium-sized compounds like fernenes are still unexplored.

Previous articles reporting correlations between experimental (δ_{exp}) and calculated (δ_{calc}) ^{13}C and ^{1}H chemical shifts in solution have used the isotropic shielding of each atom to evaluate δ_{calc}. Several authors consider the general expression:

$$\delta_{calc} = \sigma_{TMS} - \sigma \tag{1}$$

where σ_{TMS} is the isotropic shielding of tetramethylsilane, the most common experimental reference substance, calculated at the same level of theory than σ. Other substances, such as methane, have been used with this purpose [12].

It must be considered that the computations are carried out for a molecule in considerably different conditions compared to that in a real experiment, and that NMR is sensitive to short range structural changes. Therefore, large systematic differences are expected between the calculated and the experimentally measured chemical shifts. These may arise from different sources, such as electron correlation and rovibrational effects [20,32,33].

Forsyth et al. [2] reported the use of empirically scaled GIAO absolute isotropic shieldings, which have given place to other proposals. There is an extensive literature about the use of different approaches for scaling absolute shieldings [2,19,34]. An interesting work was recently carried out by Aliev et al. [34], who has reported a linear scaling of the calculated chemical shifts in order to improve the accuracy of the DFT predicted ^{13}C chemical shifts.

Additionally, the reference compound used to obtain the experimental chemical shifts must be considered. Forsyth et al., quoting other authors, have pointed the inherent problem of using a calculated shielding for a reference compound in predicting chemical shifts. Any error in the calculated shift for the single point of the reference compound will be reflected in all the derived shifts although subtracting the reference can also compensate for general discrepancy in the magnitude of the predicted absolute shielding.

However, the experimental chemical shift in "ppm" is a magnitude calculated in relation to a reference substance. Therefore, any expression that relates the experimental chemical shift with the chemical shielding tensor descriptors expressed in "ppm" (as obtained from Gaussian 03) must include a term which takes into account the reference magnitudes.

If we assign the value -1 to the scaling factor "m" and the value σ_{TMS} to the intersection in the equation proposed by Forsyth et al.:

$$\delta_{\mathrm{calc}} = m.\sigma + i \tag{2}$$

expression (**2**) reduces to (**1**).

The equation proposed by Forsyth et al. [2] is the most general form for these relationships. The proposal by Aliev et al. [34] can be reduced to a similar form considering that the scaling factor 0.95 is equal to $-m$.

The use of isotropic shielding to correlate with experimental chemical shifts seems to be absolutely reasonable considering the orientational averaging of magnetic properties when NMR experiments are carried out in solution. Our hypothesis is that it is also reasonable to consider the other parameters derived from the shielding tensor to take into account the anisotropy which can not be eliminated by orientational averaging. With this purpose, we propose to include the chemical shifts anisotropy asymmetry, η:

$$\delta_{\mathrm{calc}} = M.(\sigma + \eta) + I \tag{3}$$

In the same way like in Eq. (2), the scaling factor "M" and the interception "I" can be obtained from a linear regression between the experimental chemical shifts of a significant number of C atoms, and their respective calculated values of isotropic shielding and asymmetry.

Table 2 shows the values obtained for the parameters of the three equations used here, in different conditions: (a) calculations in vacuum, (b) calculations in the presence of solvent, (c) considering only the least energy conformer and (d) considering different conformers (see Section 3.2). All calculations were carried out by using B3LYP/6-31G(d)//B3LYP/6-31G(d) calculations.

The results summarized in Table 2 clearly show that the inclusion of a scaling factor (m) enhanced the performance of theoretical calculations, decreasing rms in approximately 37% (compare the results obtained from Eqs. (1) and (2)). Using Eq. (3) instead of Eq. (1) for GIAO calculations, the correlation was improved diminishing rms in approximately 42% (compare results from Eqs. (1) and (3)).

3.2. The conformational problem (weighting the different conformers)

The conformational mobility possessed by the fernenes reported in this work becomes a challenging aspect when making practical use of computed ^{13}C chemical shifts. Therefore, a preliminary conformational analysis was performed for the different compounds reported here. It should be noted that in general all fernenes reported here displayed conformers adopting a characteristic "cylindrical" shape (Fig. 2).

The conformational study was carried out in two steps: first, the Monte Carlo method implemented in Spartan 04® [35], using molecular mechanics (MMFF94) calculations was used in order to obtain the probable conformers of each compound; and second, from the output of this procedure the conformers considered significantly different were selected. The number of these conformations obtained for each molecule is shown in Table 3. The criteria chosen to consider a significantly different conformer were an energy gap (calculated with MMFF94) higher than 1.25 kcal/mol as well as different spatial ordering of heavy atoms (Table 3, 2nd column). Once the different conformations were obtained, the geometry of each conformer was optimized using B3LYP/6-31G(d) computations. Thus, simple geometry optimizations followed by the frequency and chemical shift calculations were performed using the same level of theory.

One approach to weighting the contributions of different conformers is to use the relative energies from the DFT calculations. The contribution of the different conformers was weighted using the Boltzmann distribution:

$$N_j = \frac{\exp(-E_j/RT)}{\sum_i \exp(-E_i/RT)}$$

Energy differences between conformational states are related thermodynamically to their populations, with a ratio of 1.10 for a free energy difference of about 1.4 kcal/mol at room temperature. Consequently, conformations with relative free energy larger than 2 kcal/mol are populated to a very minor extent. Therefore, only conformations possessing less than 2.2 kcal/mol above the lowest-energy form of each compound were considered in the GIAO calculations (Table 3, 4th column). To confirm that significant contributions were not missing when using such approach, some GIAO

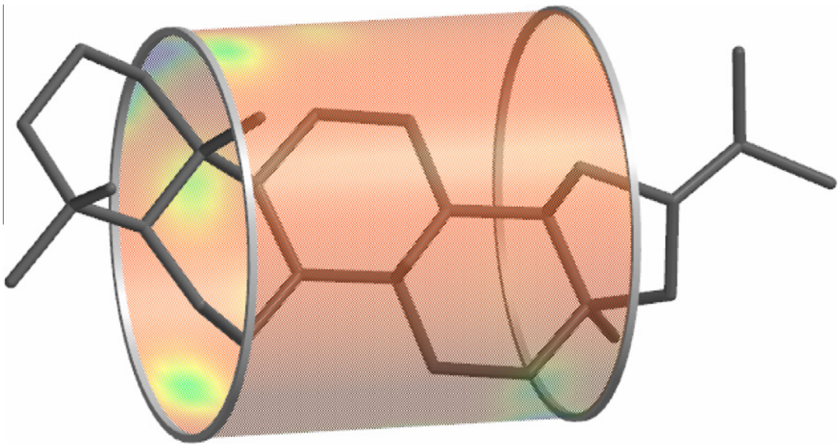


Fig. 2. Spatial view of the preferred conformation obtained for compound **1** (all the fernenes reported here displayed similar conformers). The spatial orientation of the cylinder is shown in this figure.

Table 3
Different conformers considered for each molecule from both molecular mechanics and DFT calculations.

Compound	N° of significantly different conformers (MMFF94)	Conformer	*E* (u.a) B3LYP/6-31G(d)	Δ*E* (kcal/mol) B3LYP/6-31G(d)
1	4	1-1	−1173.296	0.000
		1-2	−1173.293	2.051
2	5	2-1	−1361.859	0.000
		2-2	−1361.859	0.031
3	4	3-1	−1173.302	0.000
		3-2	−1173.299	2.140
4	3	4-3	−1248.512	0.000
		4-1	−1248.511	0.628
		4-2	−1248.511	0.628
5	8	5-2	−1361.867	0.000
		5-1	−1361.866	0.628
6	4	6-2	−1397.742	0.000
		6-1	−1397.740	0.934
7	4	7-3	−1397.737	0.000
		7-1	−1397.737	0.213
		7-2	−1397.736	0.534
		7-4	−1397.735	0.889
8	4	8-2	−1396.548	0.000
		8-3	−1396.548	0.000
		8-1	−1396.548	0.217
9	4	9-1	−1173.299	0.000
		9-2	−1173.296	2.068
10	5	10-1	−1322.555	0.000
		10-2	−1322.555	0.013
		10-3	−1322.552	2.054
11	7	11-2	−1361.863	0.000
		11-1	−1361.863	0.095
		11-3	−1361.859	2.576
12	4	12-1	−1323.717	0.000
		12-2	−1323.714	2.006
		12-3	−1323.714	2.032
13	9	13-1	−1398.923	0.000
		13-2	−1398.922	0.469
		13-4	−1398.921	0.686
		13-3	−1398.920	1.841
14	12	14-1	−1512.287	0.000
		14-3	−1512.284	1.898
15	4	15-1	−1248.505	0.000
		15-2	−1248.501	2.034

calculations were performed in parallel, considering all the conformers for compounds **1** and **15**. These results did not show any statistical difference supporting the energy window chosen.

Comparing the results obtained by weighting the contribution of different conformers or considering only the energetically preferred form (Table 3), it is clear that only a slight enhancement was obtained weighting different forms for all the evaluated compounds. This is an expected result considering the restricted conformational flexibility displayed for all the fernenes reported in this work.

3.3. Solvent effects

It is well known that conformations as well as GIAO calculations determined in gas phase are not similar to those determined in solution since environmental effects may play a significant role in determining conformations in solution. Thus, at this stage of our work we assume that solvent effects might change these results somewhat. To verify this assumption we attempted to add this effect to the computations. The PCM (polarizable continuum model) method [36] implemented in the Gaussian 03 was adopted, in which the cavity of a solute is defined by the van de Waals surface. It is clear that there are more acute approaches to consider the solvent effects in these calculations. Thus, we do not expect that the entire solutions behaviour of fernenes be explained by such a reduced treatment. The aim of these calculations is less ambitious. We intend to obtain a reasonable indication of the direction and magnitude of changes in conformational preferences and GIAO calculations of the isolated molecule when it enters chloroform solution. In addition, this approach might be directly applied into the calculations from the Gaussian's protocols and, therefore, it has the advantage to be useful for routine calculations, which is the main purpose of this work. From this point of view, the inclusion of PCM model in the computations should be particularly significant. Thus, calculations were performed in gas phase and also in the presence of chloroform and deuterated chloroform simulated in a similar way, with the purpose of comparing with experimental results which were determined in the mentioned solvents.

In general, calculations considering the solvent effects give better correlations in comparison to those obtained in gas phase (compare both results in Table 2). However, these enhancements might be considered only as moderated. It should be noted that the empirical scaling factors are considering, at least in part, the effects of solvents. Thus, it appears reasonable that the inclusion of the PCM model in the calculations gives only a limited enhancement in these correlations.

3.4. Correlations between δ_{exp} and δ_{calc} obtained from the different equations

Fig. 3a shows graphically the correlation obtained between δ_{exp} and $(\sigma + \eta)$, in solution and weighting the different conformers with Boltzmann proportion, for all compounds shown in Fig. 1 except for rubiatriol, which is discussed in detail in Section 3.5.

The correlation between δ_{exp} and δ_{calc} can be expressed as:

$$\delta_{\text{exp}} = a.\delta_{\text{calc}} + b \tag{4}$$

where "*a*" and "*b*" give a valuation about the coincidence between the experimental and the calculated chemical shifts, being $a = 1$ and $b = 0$ the ideal case.

Fig. 3b shows the correlation obtained between δ_{exp} and δ_{calc} from Eq. (3), in solution and weighting the different conformers with the Boltzmann proportion. This figure was plotted for all the compounds shown in Table 1 except for rubiatriol. The statistical descriptors of the correlation δ_{exp} vs. δ_{calc} in different conditions and using the different equations are also shown in Table 2.

Comparing the results obtained from Eqs. (1) and (2), it is clear that the inclusion of a scaling factor (m) enhanced the performance of theoretical calculations decreasing rms in approx. 37%. The use of Eq. (3) instead of Eq. (1) for GIAO calculations improves the correlation, diminishing rms in approx. 42%. When using Eq. (1), the inclusion of Boltzmann weighted conformers diminishes the rms in only 0.34% in vacuum. This inclusion increases rms in 0.23% in solvent. When using Eqs. (2) and (3), rms decreases when Boltzmann weighted conformers were included. The inclusion of solvent as a factor decreases rms in approx. 1–2%.

On the basis of our results, we can conclude that the inclusion of a scaling factor is necessary in order to obtain a good correlation between δ_{calc} and δ_{exp}. Also, the inclusion of asymmetry improves this correlation significantly. In contrast, taking into account the different conformations and the solvent effects only a slight enhancement was obtained in this case.

In general η has not been included in the equations to predict NMR chemical shifts. However, it should be noted that the mayor

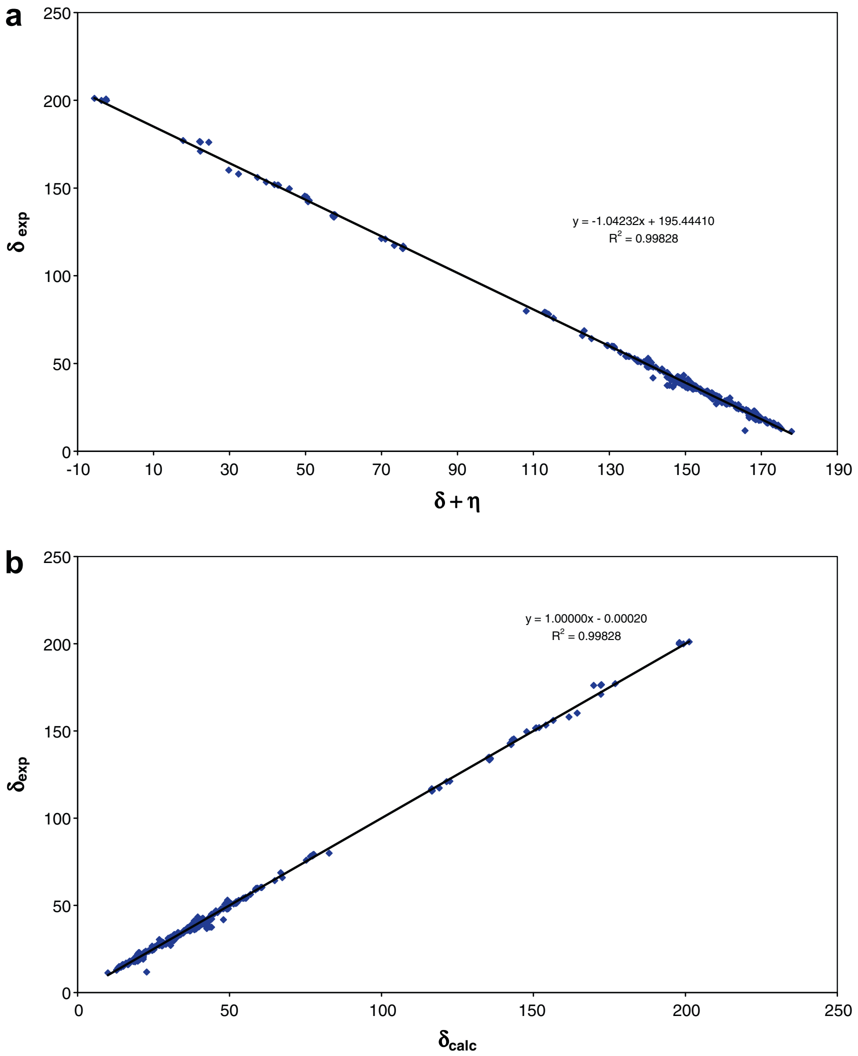


Fig. 3. (a) Plot of experimental ^{13}C chemical shifts vs. theoretical $\sigma + \eta$ from GIAO B3LYP/6-31G(d)//B3LYP/6-31G(d) calculations for the 14 organic compounds in Table 1 (compound **13** (rubiatriol) was not included and is discussed in detail in the next section). (b) Plot of δ_{exp} vs. δ_{calc} calculated using Eq. (3), in solution, and for all compounds except rubiatriol.

or minor effect of this inclusion could be directly related with the overall spatial ordering adopted by the molecule. In other words anisotropy would be of lesser importance for those molecules adopting a "spherical" shape. By spherical shape we mean spatial orderings symmetrical considering any direction. In contrast, in the case of molecules adopting non spherical orderings, which are not symmetrical considering different directions, anisotropy could be significant for these calculations. All the fernenes reported here displayed a "cylindrical" shape and therefore it appears that the inclusion of η in these calculations produce an enhancement in the correlations between the calculated and experimental results.

3.5. The case of rubiatriol. Are the ^{13}C NMR chemical shifts of this compound well assigned?

As shown in Section 3.4, an excellent correlation between experimental and DFT/GIAO computed ^{13}C NMR chemical shifts was obtained for the 14 fernenes analyzed in this report. In contrast we have serious problem with rubiatriol (compound **13**). In fact there is not any correlation between experimental and DFT/GIAO computed ^{13}C NMR chemical shifts for this compound. Considering the possibility that our calculations possess some problem to reproduce the experimental data of rubiatriol [37], the analysis of this compound was also performed employing two different commercially available software packages (ACD [38] and MestreNova Predictor [39]). These results were even worse than those obtained by using DFT/GIAO calculations (Fig. 4). Interestingly, our calculations using Eq. (3) displayed a lower rms with respect to both commercial packages for the rest of the fernenes as well (data not shown). On the basis of these results the questions which arise are: is there any problem to calculate the ^{13}C NMR chemical shifts of rubiatriol? Is the structure of this compound particularly complex or structurally special to avoid such calculations? Or is there any problem or mistake with the experimental ^{13}C NMR chemical shifts assignments? It should be noted that compound **12** is structurally very similar to rubiatriol. However, the correlation obtained for that compound was excellent, showing one of the best correlations in this series (see Fig. 5). Observing the structure of rubiatriol in detail, there is not *a priori* any complexity related with its structural feature. On the basis of such situation an exhaustive search about previous citations on rubiatriol was performed. It is interesting to note that this structure was reported only once by Arisawa et al. [37], using a 200 MHz NMR. All the subsequent citations (in fact we only found very few) were referred to the original report. Thus, we had serious and reasonable doubts about the ^{13}C NMR chemical shifts originally assigned to rubiatriol.

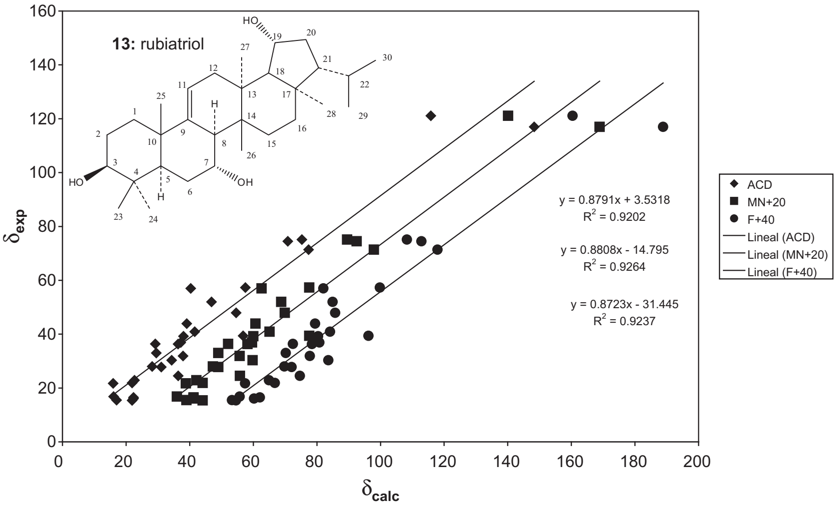


Fig. 4. Plot of experimental ^{13}C chemical shifts (δ_{exp}) vs. δ_{calc} from GIAO B3LYP/6-31G(d)//B3LYP/6-31G(d) calculations for compound **13** (rubiatriol). Calculations were performed using Eq. (3) (F), ACD/CNMR (ACD), and MestreNova® (MN). It should be noted that for MN and F arbitraries values (20 and 40 units) were added to separate the data.

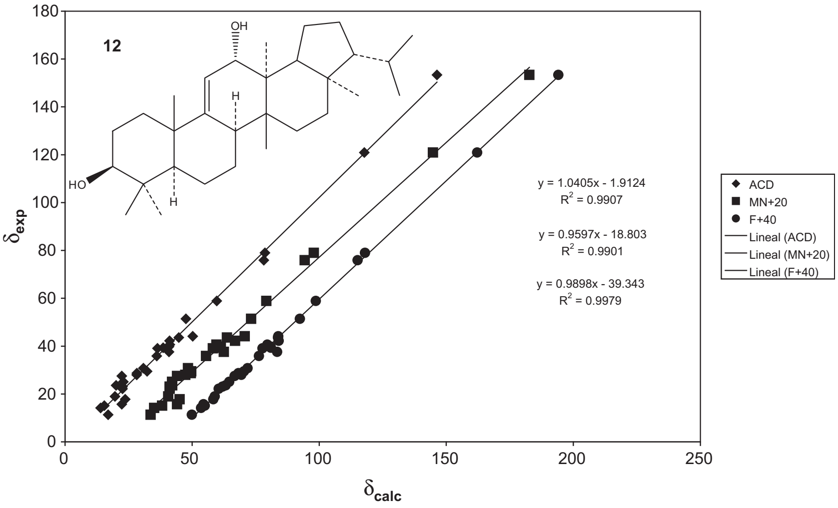


Fig. 5. Plot of experimental ^{13}C chemical shifts (δ_{exp}) vs. δ_{calc} from GIAO B3LYP/6-31G(d)//B3LYP/6-31G(d) calculations for compound **12**. Calculations were performed using Eq. (3) (F), ACD/CNMR (ACD) and MestreNova (MN). It should be noted that for MN and F arbitraries values (20 and 40 units) were added to separate the data.

It must be pointed out that the main purpose of this study is to obtain advances in the correlation between experimental and DFT/GIAO computed ^{13}C NMR chemical shifts, trying to maintain the level of calculation as reasonable as possible. However, considering our results, we believe that the previously reported ^{13}C NMR chemical shifts of rubiatriol have been called into question, and therefore, it appears necessary to corroborate this spectrum by using more accurate experimental methods.

These results indicate that DFT/GIAO computations reported in this work might be an excellent complement for the experimental ^{13}C NMR analysis of this kind of compounds, avoiding mistakes and erroneous assignments.

4. Conclusions

The ^{13}C NMR chemical shifts of 15 pentacyclic terpenoids were calculated using the DFT/GIAO method. In our strategy of using a small basis set for GIAO calculations along with non-expensive DFT calculations to achieve maximum practicality, the best results (considering a reasonable cost-to-benefit ratio) were obtained from B3LYP/6-31G(d)//B3LYP/6-31G(d) computations.

The calculated ^{13}C NMR chemical shifts of the fernenes reported here strongly agree with the available experimental values. In this sense it appears that by enhancing the extension of the basis set used in these calculations, it is difficult to expect a significant improvement on these results. Isotropic ^{13}C NMR shieldings from GIAO calculations account well for relative chemical shifts even with relatively modest basis sets, as long as the effects of electron correlation are included via the DFT approach. However, the GIAO shieldings for the basis sets examined in this paper require empirical scaling to give good numerical agreement with experimental chemical shifts. Additionally, we introduced a combination of shielding parameters instead of a unique one for the first time in this type of calculations. This parameter is related to the asymmetry, and its inclusion in the calculations gives a better correlation with the experimental data.

Previously, Forsyth et al. proposed “to achieve the goal of routine practical use, predicted ^{13}C chemical shifts need to be accurate to within a very few ppm for molecules in solution that include a wide variety of functional groups and conformational characteristics. The predictions also need to be achieved at modest computational cost". It should be noted that our results satisfy both premises. Only differences of 1.5 ppm were observed between our calculations and the experimental data. Also, it should be emphasized that our calculations were carried out using B3LYP/6-31G(d) calculations which demand a quite reasonable computational requirement. Our approach has worked very well on fernenes reported here. However, the appropriateness of this protocol should be tested carefully for all other structural types.

Acknowledgements

This work was supported by grants from Universidad Nacional de San Luis. R.D.E. is a member of the CONICET (Argentina) staff.

References

[1] (a) P.v.R. Schleyer, J.W. de M. Carneiro, J. Am. Chem. Soc. 112 (1990) 4046;
(b) M. Bühl, P.v.R. Schleyer, J. Am. Chem. Soc. 4 (1992) 1.
[2] D.A. Forsyth, A.B. Sebag, J. Am. Chem. Soc. 119 (1997) 9483–9494.
[3] (a) E. Koglina, E.G. Wittea, S. Willboldb, R.J. Meier, J. Mol. Struct. (THEOCHEM) 712 (2004) 233–237;
(b) A. Plaza, S. Piacente, A. Perrone, A. Hamed, C. Pizza, G. Bifulcoa, Tetrahedron 60 (2004) 12201–12209;
(c) A. Plaza, A. Perrone, C. Balestrieri, M.L. Balestrieri, G. Bifulco, V. Carbone, A. Hamed, C. Pizza, S. Piacente, Tetrahedron 61 (2005) 7470–7480;
(d) K. Tuppurainen, J. Ruuskanen, Chemosphere 50 (2003) 603–609;
(e) A. Balandina, V. Mamedov, X. Franck, B. Figadère, S. Latyp, Tetrahedron Lett. 45 (2004) 4003–4007;
(f) A.I.R.N.A. Barros, A.M.S. Silva, I. Alkortac, J. Elguero, Tetrahedron 60 (2004) 6513–6521.
[4] T. Onak, T.J. Tran, D. Herrera, B. Chan, J. Arias, M. Diaz, Inorg. Chem. 31 (1992) 3910.
[5] (a) M. Bühl, J. Gauss, M. Hofmann, P.v.R. Schleyer, J. Am. Chem. Soc. 115 (1993) 12385;
(b) A. Rauk, T.S. Sorensen, C. Maerker, J.W. de M. Carneiro, S. Sieber, P.vR. Schleyer, J. Am. Chem. Soc. 118 (1996) 3761.
[6] (a) D. Jiao, M. BarField, V.J. Hruby, Mag. Reson. Chem. 31 (1993) 75;
(b) A.C. de Dios, J.G. Pearson, E. Oldfield, Science (Washington, DC) 260 (1993) 1491;
(c) A.C. de Dios, E. Oldfield, Chem. Phys. Lett. 205 (1993) 108;
(d) A.C. de Dios, J.C. Pearson, E. Oldfield, J. Am. Chem. Soc. 115 (1993) 9768;
(e) A.C. de Dios, E. Oldfield, J. Am. Chem. Soc. 116 (1994) 5307;
(f) H.M. Sulzbach, P.v.R. Schleyer, H.F. Schaefer III, J. Am. Chem. Soc. 117 (1995) 2632;
(g) Y. He, D. Wu, L. Shen, B. Li, Mag. Reson. Chem. 3 (1995) 701.
[7] (a) M. Wada, M. Sakurai, Y. Inoue, R. Chujo, Mag. Reson. Chem. 30 (1992) 831;
(b) M. Wada, M. Sakurai, Y. Inoue, Y. Tamura, Y. Watanabe, J. Am. Chem. Soc. 116 (1994) 1537;
(c) M. Wada, M. Sakurai, Y. Inoue, Y. Tamura, Y. Watanabe, Mag. Reson. Chem. 33 (1995) 453;
(d) H. Houjou, M. Sakurai, N. Asakawa, Y. Inoue, Y. Tamara, J. Am. Chem. Soc. 118 (1996) 8904.
[8] (a) W. Kutzelnigg, Isr. J. Chem. 19 (1980) 193;
(b) M. Schindler, W. Kutzelnigg, J. Am. Chem. Soc. 104 (1983) 1360.
[9] (a) A.E. Hansen, T.D. Bouman, J. Chem. Phys. 82 (1984) 5035;
(b) A.E. Hansen, T.D. Bouman, J. Chem. Phys. 91 (1989) 3552.
[10] (a) R. Ditchfield, Mol. Phys. 27 (1974) 789;
(b) C.M. Rohling, L.C. Allen, R. Ditchfield, Chem. Phys. 87 (1984) 9;
(c) K. Wolinski, J.F. Hinton, P. Pulay, J. Am. Chem. Soc. 112 (1990) 8251.
[11] T.A. Keith, R.F.W. Bader, Chem. Phys. Lett. 210 (1993) 223.
[12] J.R. Cheeseman, G.W. Trucks, T.A. Keith, M.J. Frisch, J. Chem. Phys. 104 (14) (1996) 5497.
[13] T.M. Alam, Ab Initio Calculation of Nuclear Magnetic Resonance Chemical Shift Anisotropy Tensors. I. Influence of Basis Set on the Calculation of ^{31}P Chemical Shifts, Sandia National Laboratories, 1998.
[14] (a) J. Gauss, J. Chem. Phys. 99 (1993) 3629;
(b) M. Kollwitz, J. Gauss, Chem. Phys. Lett. 260 (1996) 639;
(c) S.M. Cybulski, D.M. Bishop, J. Chem. Phys. 106 (1997) 4082.
[15] S.B. Mahato, A.P. Kindu, Phytochemistry 37 (6) (1994) 1517–1575.
[16] A.M.L. Seca, A.M.S. Silva, A.J.D. Silvestre, J.A.S. Cavaleiro, F.M.J. Domingues, C.P. Neto, Phytochem. Anal. 11 (2000) 345–350.
[17] O. Christiansen, J. Gauss, J.F. Stanton, Chem. Phys. Lett. 266 (1997) 53.
[18] C. van Wullen, W. Kutzelnigg, J. Chem. Phys. 104 (1996) 2330.
[19] Y. Zhang, A. Wu, X. Xu, Y. Yan, J. Phys. Chem. A 111 (2007) 9431.
[20] A. Wu, Y. Zhang, Y. Yan, J. Comput. Chem. 28 (2007) 2431.
[21] G. Magyarfalvi, P. Pulay, J. Chem. Phys. 119 (2003) 1350.
[22] S.N. Maximoff, J.E. Peralta, V. Barone, G.E. Scuseria, J. Chem. Theory Comput. 1 (2005) 541.
[23] T.W. Keal, T. Helgaker, P. Salek, D.J. Tozer, Chem. Phys. Lett. 425 (2006) 163.
[24] K.W. Wiitala, T.R. Haye, C.J. Cramer, Chem. J. Theory Comput. 2 (2006) 1085.
[25] Y. Zhao, D.G. Truhlar, J. Phys. Chem. A 112 (2008) 6794.
[26] Y. Yang, S.X. Huang, Q.S. Zhao, J. Phys. Chem. A 112 (2008) 12132.
[27] A.D. Becke, J. Chem. Phys. 98 (1993) 5648.
[28] J.C. Slater, Quantum theory of molecules and solids, The Self-Consistent Field for Molecules and Solids, vol. 4, McGraw Hill, New York, 1974.
[29] A.D. Becke, Phys. Rev. B 38 (1988) 3098.
[30] S.H. Vosco, L. Wilk, M. Nusair, Can. J. Phys. 58 (1980) 1200.
[31] C. Lee, W. Yang, R.G. Parr, Phys. Rev. B 37 (1988) 785.
[32] K.K. Baldridge, J.S. Siegel, J. Phys. Chem. A 103 (1999) 4038.
[33] D.B. Chesnut, Chem. Phys. Lett. 214 (1997) 73.
[34] A.E. Aliev, D. Courtier-Murias, S. Zhou, J. Mol. Struct. (THEOCHEM) 893 (2009) 1–5.
[35] SPARTAN'04, Wavefunction, Inc., Irvine, CA.
[36] S. Miertus, E. Scrocco, J. Tomasi, Chem. Phys. 55 (1981) 117.
[37] M. Arisawa, H. Ueno, M. Nimura, T. Hayashi, N. Morita, J. Nat. Prod. 49 (1986) 1114–1116.
[38] ACD/CNMR®, v2.51, 1997.
[39] MestreNova, NMRPredict Desktop®, 2008.