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Can Two Molecules Have the Same NMR Spectrum? Hexacyclinol Revisited

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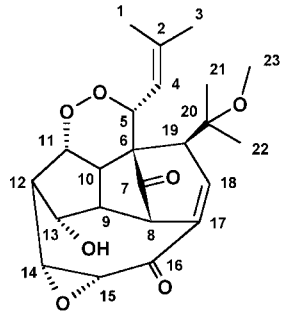
ABSTRACT



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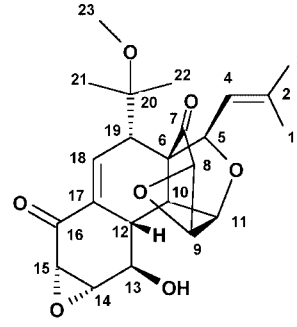

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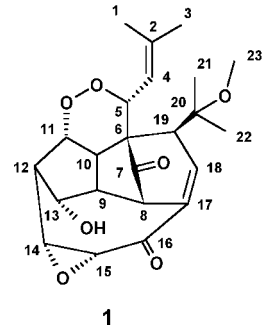
An in-depth DFT computational investigation (B97−2/cc-pVTZ level) of the ^{1}H and ^{13}C NMR spectra of the recently disputed natural substance hexacyclinol, including $J(^{1}H,^{1}H)$ couplings, is presented. Structures 1 and 2 have been compared with regard to the suggested possibility that two molecules have very similar NMR spectra as to be indistinguishable. Despite a remarkable similarity of functional groups present, the two calculated spectra differ in many features related both to chemical shifts and connectivities.

Nature continues to surprise us with a staggering variety of chemical structures. Almost always, isolation and identification of such substances takes advantage of a wide array of spectroscopic techniques, among which NMR spectroscopy plays a distinctive role. Even the huge amount of information thereby provided,[1] however, may not lead to an unambiguous structure elucidation, as presented by Nicolaou and Snyder in their review about the "Molecules That Were Never There".[2]

The "hexacyclinol dispute" fits precisely into this category. The debate spurred after the isolation in 2002 of a novel complex molecule from the basidiomycete *Panus rudis*,[3] for which structure **1** was proposed on the basis of NMR results (Scheme 1). Following its promising biological activity, a total synthesis was realized by La Clair[4] and subsequently challenged by Rychnovsky[5] and Porco[6] on the basis of two

Scheme 1. Structures of Hexacyclinol Proposed by La Clair (**1**) and Rychnovsky (**2**)



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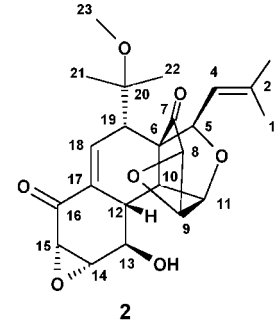

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very different arguments: Rychnovsky suggested an alternative structure (**2**) and used calculated ^{13}C NMR chemical shifts to establish the best match between the two data sets, finding a much better agreement for **2**, which was also thought to be favored from biosynthetic considerations.

Shortly thereafter, Porco and co-workers presented a total synthesis and X-ray crystal structure of **2**, which exhibited NMR spectra identical to the original ones. Finally, La Clair postulated that he and Porco may have made two different molecules that happen to have very similar NMR spectra.[7]

Indeed, compelling as Porco's evidence is, the NMR spectral assignment of hexacyclinol remains far from trivial.

On one hand, Rychnovsky's computational analysis employed geometries calculated at the Hartree−Fock (HF) level of theory and was limited to ^{13}C NMR; no attempt was made at predicting ^{1}H spectra including shifts and couplings, which provide a rich source of spectral signatures. On the other hand, NMR spectral predictions based on expert systems have proved difficult: Williams et al. had to make a large number of assumptions in order to distinguish the spectra of **1** and **2**.[8]

Thus, it seems that there are still significant gaps in our understanding of these spectra; indeed, the possibility that two molecules as complex as **1** and **2** may have indistinguishable NMR spectra carries an uneasy feel. However, our previous experience with complex natural substances led us to believe that advanced theoretical methods may provide a novel framework for assessment.

Density functional methods (DFT), which partly include electron correlation, are recommended for the calculation of chemical shifts, and mandatory for coupling constants.[9] We have previously shown that an accuracy of ca. 0.1 and 1 ppm, respectively, can be attained for calculated ^{1}H and ^{13}C shifts of a variety of organic molecules, including naturally occurring ones.[10−12] Hence, we have deployed state-of-the-art computational tools to predict the basic NMR spectral features of the conflicting structures **1** and **2**, that is, ^{1}H shifts and homonuclear couplings.

The structure of **1** and **2** was optimized at the B3LYP/6−31G(d,p) level, in accordance with previous works.[10−12] NMR shifts and J(H,H) coupling constants were then computed at the B97−2/cc-pVTZ level.[13] The B97−2 exchange-correlation functional has recently been shown to yield good quality NMR parameters, especially spin−spin coupling constants.[14,15] All calculations were carried out with Gaussian 03.[16] Because the molecule is fairly rigid, there is no need to search the potential energy surface for conformers. Also, because experimental data have been collected in $CDCl_3$, negligible solvent effects are expected.

Following Rychnovsky,[5] we have considered two conformers of **2**, differing in the rotation across the C4−C5 bond. The two resulting structures (**2a** and **2b**) differ by ca. 0.6 kcal/mol, that is, within the typical error of DFT methods; for this reason, their properties were averaged.[17] In our comparison, we do not consider the hydroxyl proton since its chemical shift is strongly affected by traces of water.

A statistical analysis of ^{1}H shifts for **1** leads to a mean absolute error (MAE) of 0.4 ppm, whereas the MAE for **2** is much smaller (0.2) (Figure 1; details in Tables S1−S2, Supporting Information). In particular, proton H9 (which has quite different environments in **1** and **2**) is now in much better agreement with the experimental values.

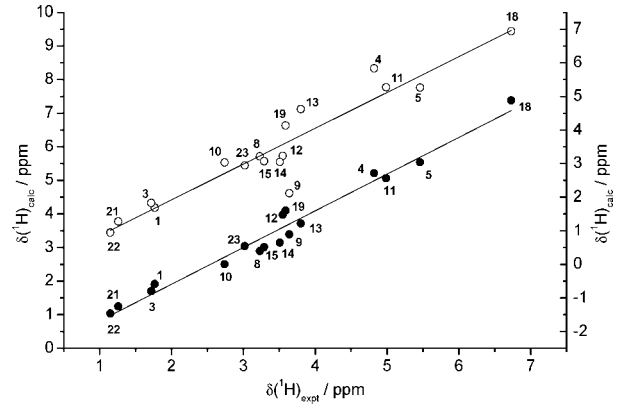

Figure 1. Correlation between calculated and experimental ^{1}H chemical shifts. ○, right axis (shifted by 2.5 ppm for clarity: **1**. ●, left axis: average of structures **2a** and **2b**. Solid lines are the best fit to $\delta_{calc} = a + b\,\delta_{exp}$. **1**: $a = -0.22$ ppm, $b = 1.066$, $r^2 = 0.89$; **2**: $a = -0.28$ ppm, $b = 1.093$, $r^2 = 0.97$. See Scheme 1 for label assignments.

Correlation of ^{13}C data yielded very similar results to those obtained by Rychnovsky (Figure S2, Supporting Information) and will not be detailed.

Computed J(H,H) couplings for **1** (Figure 2) show a rather poor agreement with experiment, whereas a very good correlation is observed for **2** (except for ^{3}J(H4,H5), whose value strongly depends on the conformation of the olefinic group).

In particular, in La Clair's assignment H14 (3.51 ppm) is a doublet of doublets with small ^{3}J coupling constants (ca. 3.0 and 0.5 Hz). However, even from simple Karplus arguments one would expect a large value of ^{3}J with H15

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(17) The NMR parameters of **2a** and **2b** are quite similar, except for ^{3}J(H4,H5) and methyl proton H1. However, the conformation of the olefinic moiety is not a critical issue.

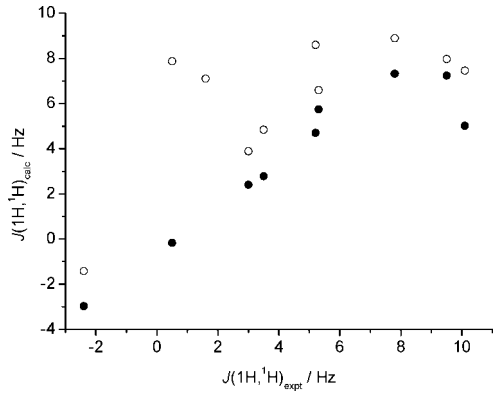

Figure 2. Correlation between calculated and experimental ^{1}H,^{1}H coupling constants. ○: **1**; ●: average of structures **2a** and **2b**. The negative sign of the experimental ^{4}J(H18,H12) is deduced from the calculated results.

and H12, since for **1** the dihedral angle is close to zero in both cases; the calculated values are, in fact, 3.9 and 7.9 Hz, respectively.

Likewise, H13 (3.81 ppm) is a doublet of doublets with a large and a small coupling constant (9.5 and 1.6 Hz), while the orientation of H13 with respect to H12 and H9 again features a dihedral angle of about zero in both cases, and both calculated coupling constants are large (8.0 and 7.1 Hz, respectively).

In contrast, in **2** the corresponding H14−H13 dihedral angle is close to 90°, thus justifying the observed and calculated small ^{3}J between them (Table S3, Supporting Information).

In summary, there is hardly any doubt that **2** is the correct structure for hexacyclinol. We can now turn to our question, can the structures **1** and **2** yield so similar NMR spectra as to be virtually indistinguishable as suggested?[7] The case in point is indeed subtle, since the two structures share many structural features and functionalities (Scheme 1).

Common features are: (a) Two pairs of methyl (H1,H3; H21,H22) and a methoxy (H23) group. H21−H23 are uncoupled with the rest of the spin system; H1 and H3 appear as singlets but cross-peaks are present in COSY spectra (weak couplings of ca. 1 Hz are in fact calculated). (b) Two endocyclic olefin protons (H4, H18) and one CH(OH) methine proton (H13).

On the contrary, **1** has two CH protons bound to a peroxide (H5, H11), which in **2** are replaced by CH−O(ether ring). Also, five aliphatic CH (H8, H9, H10, H12, H19) in **1** are only partly retained in **2** (H10, H12, H19) since **2** possesses two epoxide rings (H8, H9; H14, H15) rather than one.

Considering ^{1}H shifts, predictably the largest difference between **1** and **2** is observed for H9 (Figure S4, Supporting Information). Yet, smaller but comparable differences are found for H13, H12 and H4 even though they belong to the same functional group (CH(OH), aliphatic CH and olefin, respectively), whereas the ether and peroxide protons (H5, H11) do not differ much.

With regard to ^{13}C shifts (Figure S4, Supporting Information), C5 and C11 (ether and peroxide CH) now show the largest difference. However, as before, there are large discrepancies for C19, C10, C16, and C7 despite the same functionality (aliphatic CH and C═O).

Therefore, functional similarity is not only insufficient to ensure NMR spectra are similar, but may even be a misleading criterion. When one adds the information from

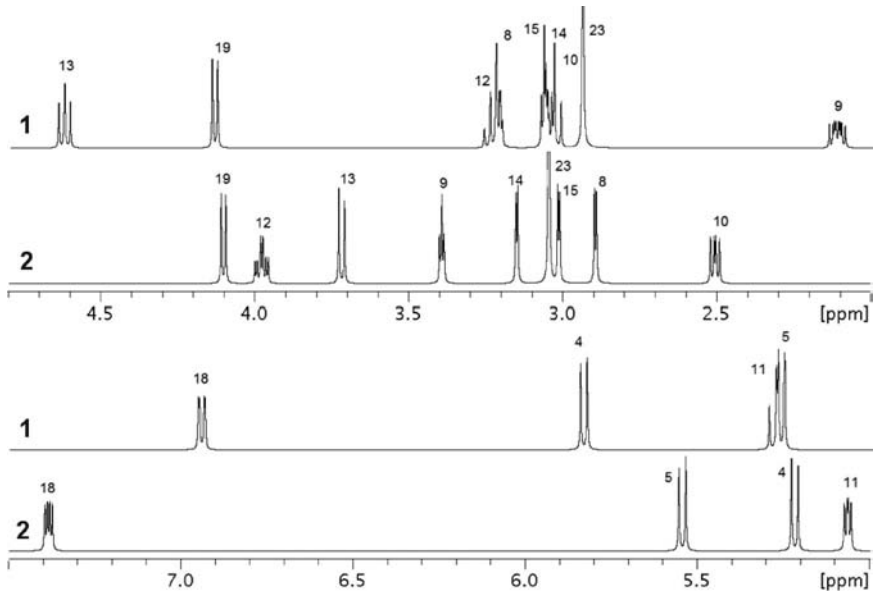

Figure 3. Calculated ^{1}H spectra of hexacyclinol, split into two regions for clarity. The 0−2 ppm region is not included because it only contains methyl signals that appear as singlets even though cross-peaks are present in COSY spectra (full data in Figure S8, Supporting Information).

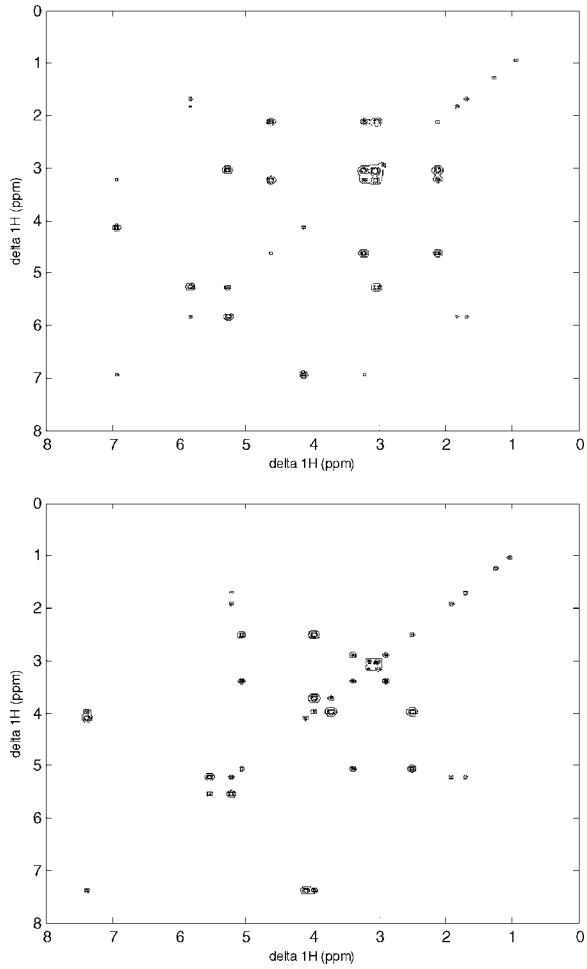

Figure 4. Simulated COSY spectra of **1** (top) and **2** (bottom).

spin−spin couplings, as detailed above, the picture becomes even clearer. Calculated spectra of **1** and **2**, including multiplicities, are compared in Figure 3.[18] This presentation effectively conveys at a glance the desired information, that is, the degree of similarity between the spectra of the two molecules. Apart from the 0−2 ppm region, which only contains methyl singlet peaks (Figure S8a, Supporting Information), the other regions clearly show different coupling patterns. The different connectivity patterns can be further visualized by comparing simulated COSY spectra[18] in Figure 4.

We can summarize these results as follows. DFT calculations are a fast, affordable and reliable means to predict the NMR spectra (chemical shifts and couplings) of unknown molecules, which confirms the viability of this approach also in this difficult case. We remark that no empirical assumption was made concerning the resonance frequencies and connectivity patterns, which is clearly a strong point when dealing with unusual features such as uncommon heteroatoms,[12] or, as is the case here, a peroxide. The calculations have also highlighted some counterintuitive features, that is, functional similarity does not at all ensure spectral similarity.

The structure of hexacyclinol is confirmed to be **2**. Furthermore, if **1** had been synthesized or was formed from an unforeseen reaction, its NMR spectra are sufficiently different from those of **2** as to guarantee their distinction.

Clearly, it is difficult to generalize on the statement that two molecules *cannot* have the same NMR spectrum at all. Nevertheless, it seems unlikely that such an occurrence takes place, except perhaps when dealing with extremely simple or extremely crowded spectra (where information from NMR would be scarce or difficult to extract anyway).

Acknowledgment. Computational resources were provided by the Laboratorio Interdipartimentale di Chimica Computazionale (LICC) at the Department of Chemistry of the University of Padova.

Supporting Information Available: Complete ref 16, Cartesian coordinates and energies, detailed NMR parameters and spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) 1D spectra were simulated with the *nmrsim* module in Topspin 2.0 by Bruker, including strong coupling effects. 2-D COSY spectra were sketched as a superposition of 2-D Lorentzian functions at the calculated chemical shift(s). Off-diagonal peaks were added for all pairs of spins having $|J| > 1$ Hz, with an intensity scaled by a factor proportional to J. Hence, peak contours are only intended to convey qualitative connectivity information.