A Case Study of Empirical and Computational Chemical Shift Analyses: Reassignment of the Relative Configuration of Phomopsichalasin to that of Diaporthichalasin

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Abstract

Phomopsichalasin was isolated and assigned structure **1** over 15 years ago. Analysis of its proton NMR data led us to hypothesize that not all aspects of the relative configuration of this structure were correct. We have used both empirical and computational methods to propose an alternative structure. Diaporthichalasin was reported several years ago, and its structure was assigned as **7**, a diastereomer of structure **1**, and confirmed by a single crystal X-ray study. We have shown that diaporthichalasin and phomopsichalasin are identical; i.e., both have structure **7**. Additional aspects of NMR interpretation that provide guidance for avoiding some of the pitfalls that can lead to incorrect structure assignments are discussed. These recommendations/reminders include i) the use of complementary solvents for acquiring NMR data that break accidental chemical shift degeneracy, ii) the importance of assigning coupling constants as extensively as possible, and iii) exercising caution when interpreting correlations in 2D spectra where overlapping resonances are involved.

In 1995 Horn and co-workers reported the isolation of a new cytochalasin-like secondary metabolite from an endophytic fungus *Phomopsis* sp., which they named phomopsichalasin.[i] The compound exhibited antibacterial activity in several disk diffusion assays. The researchers assigned structure **1** (Scheme 1) on the basis of analysis of a battery of 1D and 2D NMR data. Phomopsichalasin represents the first compound wherein the macrocyclic portion of the cytochalasin skeleton has been cyclized to an embedded A/B/C tricyclic subunit. Diaporthichalasin (**7**) was reported in 2007 and was deduced to have a structure diastereomeric with that of **1**.[ii]

RESULTS AND DISCUSSION

The structure originally deduced for phomopsichalasin, **1** (Scheme 1),[i] is intriguing because of the possibility that it might arise via a biosynthetic pathway involving a pair of (sequential) net [4+2] intramolecular Diels-Alder (IMDA) reactions commencing from the polyene precursor **4**. The double-diene/double-dienophile **4** is a reasonable biosynthetic intermediate because it could arise via polyketide synthase (PKS) and non-ribosomal peptide synthase (NRPS) pathways. In principle, either of two IMDAs within **4**, leading either to octalin **2** or macrocycle **3**, could be the first to occur en route from **4** to **1**. We have speculated that these IMDA reactions might occur in the absence of a specific enzyme

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catalyst—that is, might one or both cycloadditions be sufficiently and inherently fast to occur spontaneously? If so, then the relative configuration of the nine non-carbinolamide stereocenters in **1** would be established by the relative asymmetric induction originating from the C-18 methyl-bearing stereocenter in **4**. However, it would be odd if **1** were the product of a substrate-induced IMDA reaction because the relative configuration between C-18 and C-16 in **1** would be unexpected. That is, the diaxial relationship between the methyl groups attached to those stereocenters is destabilizing, and the IMDA reaction leading from **4** to **2** (or **3** to **1**) would be expected to have a higher activation barrier because of the synpentane interaction partially established in the transition state geometry.

This dilemma prompted us to reexamine the data upon which the structural assignment of **1** was based. In particular, the ^{1}H NMR chemical shifts (methanol-d_4) of two protons first attracted our attention. Namely, the resonances for the axial protons at C-17 and C-19 in phomopsichalasin appear at 0.67 and 0.56 ppm, respectively. For the reasons discussed next, we surmised that these values were untenable for structure **1**.

Grant (empirical) NMR Analysis

Empirical interpretation of chemical shifts has been a mainstay of NMR analysis since the infancy of NMR spectroscopy. A methyl substituent on a cyclohexane ring is known to induce significant chemical shift perturbations on various other protons on that ring. The magnitude of some of these through-bond anisotropies is surprisingly large (Figure 1A). These shift perturbations emerged from an insightful analysis of the (natural abundance deuterium) chemical shifts of numerous methyl-substituted cyclohexanes done in the Grant laboratories.[iii] Hence, we refer to these as the "Grant numbers" and find them to be quite broadly applicable to the interpretation of chemical shift data in the context of a variety of structural settings. Quite relevant to the case of phomopsichalasin, the effect of an axially vs. equatorially oriented methyl group on the axial proton at C-2 (see gray-highlighted data in each of structures **8eq** vs. **8ax**) is particularly striking—H-2$_{ax}$ is perturbed by over 0.5 ppm simply depending upon the dihedral relationship (anti vs. gauche) of its H—*C-2 bond relative to the vicinal C-1—Me bond*! That is, when the methyl group is axial, the dihedral angle to H-2$_{ax}$ is ca. 180° and H-2$_{ax}$ is relatively deshielded, whereas when the methyl is equatorial, the dihedral is ca. 60° and H-2$_{ax}$ is shielded.

By applying the logic of a Grant analysis to structure **1**, we observed that the high field nature of the chemical shift values for H-17$_{ax}$ and H-19$_{ax}$ are inconsistent with the axial orientation of the 18-methyl substituent (cf. substructure **9a**, Figure 1B), which represents the geometry of the dominant conformer expected for the *trans*-fused AB bicyclic octalin skeleton of **1**. Instead, these shift values are much more compatible with an equatorial orientation for the 18-methyl group (cf. **9b**). The diastereomeric relationship in **9b** is a more reasonable stereochemical outcome for an IMDA reaction (forming the bold bonds in the cyclohexenyl B-ring in **9b**) proceeding under control of relative internal asymmetric induction.

We then noticed the report from Pornpakakul and coworkers in 2007 describing the isolation (from the endophytic fungus *Diaporthe* sp. Bkk3) and structure determination of diaporthichalasin.[ii] Their thorough NMR analysis efforts led to the assignment of structure **7** (Scheme 1), which was confirmed by a single crystal x-ray structure determination, placing the assignment as **7** on secure ground. Interestingly, structure **7** shares the constitution of **1**, but differs in the relative configuration of several stereocenters. The differences between structures **7** and **1** bear directly on the question of possible spontaneity of the IMDA events. That is, **7** could arise from **5a** or **6** (cf. Scheme 1), either of which could be formed from **4**. Of course, this presents a quandary, because **4** is the same polyene precursor that we earlier

identified by retrobiosynthetic analysis of structure **1**. Obviously, the uncatalyzed reaction of **4** would follow the same pathway(s) regardless of what organism may have produced that biosynthetic intermediate.

This dichotomy could be construed as evidence that the cyclization chemistry of **4** is not spontaneous but, rather, is promoted by (Diels-Alderase-like) enzymes unique to each organism. We, however and in light of this information, returned to the question of the structure assigned to phomopsichalasin—i.e., **1**. In contrast to **1**, diaporthichalasin (**7**) contains: i) an equatorial methyl substituent at C-18; ii) relative configuration of the five stereocenters within its AB bicyclic octalin subunit that is entirely consistent with the geometry expected to arise from a concerted IMDA reaction (cf. **9b** in Figure 1 and **4** to **5a** in Scheme 1); and iii) four stereocenters within its D-ring that would be anticipated from an IMDA cycloaddition in which the ketocarbonyl had been oriented endo to the C-5–C-8 diene (cf. **5a** to **7** or **4** to **6**, Scheme 1) rather than the lactam carbonyl (cf. **2** to **1** or **4** to **3**, Scheme 1).

Computational (DFT) Analyses

To further examine the hypothesis that structure **1** is an incorrect formulation for phomopsichalasin within (at least) the fused AB bicyclic subunit, we undertook a computational analysis. To make this study more tractable, we elected to examine two truncated structures that retained the ABC tricyclic substructure unit but lacked the additional complexity associated with the D- and E-rings and their substituents. Specifically, we computed ^{1}H chemical shifts for the truncated substructures **10a** and **10b** (Table 1), which correspond to the ABC tricyclic skeleton common to both structures **1** and **7**. They are tetraepimeric at carbons 16, 21, 22, and 13; that is, truncated structure **10a** has the same relative configuration within its A-ring as **1** (i.e., "phomopsichalasin-like"), and **10b** as **7** ("diaporthichalasin-like"). In each structure we maintained i) a *trans* orientation between H-21/H-22 in view of the ~12.8 Hz coupling constant between these two protons and ii) a *cis*-ring fusion between the hydrindenone substructure (i.e., across C-13/C-22) based on the observed NOE and ~8.1 Hz coupling constant between protons H-13 and H-22 in the spectrum of phomopsichalasin. Each of structures **10a** and **10b** was subjected to an initial molecular mechanics conformational search, resulting in an easily manageable number of conformational minima (two for **10a** and one for **10b**). DFT geometry optimization with the M062X functional[iv] [6–31+G(d,p) basis set] was performed on each of these conformers. Finally, chemical shift calculations were carried out using our recently developed WP04 functional,[v] the polarizable continuum model (PCM) for MeOH solvation, and the pcS-2 basis set.[vi]

The computed (and Boltzmann weighted) proton chemical shifts (δ_{DFT}) for **10a** and **10b** are listed in Table 1, as are the experimental shifts[i] reported for the analogous protons in the ^{1}H NMR spectrum of phomopsichalasin (methanol-d_4, δ_{EXP}). The differences between the computed and experimental shift values ($|\Delta\delta|$) are listed in the rightmost pair of columns. The bottom-line is presented as the mean absolute error (MAE) value for each of the comparisons of **10a** vs. "**1**" vis-à-vis **10b** vs. "**1**" (we use "**1**" here as a shorthand notation for "the spectroscopic data reported for phomopsichalasin"). Using the full set of all 14 numbered protons (MAE_{FULL}, cf. the structure of **10a**), substructure **10b** clearly shows a substantially better match than **10a** between the calculated and experimental data sets (**0.11** vs. **0.25**, respectively). If instead only the subset of protons that reside nearest to the point of difference in the relative configuration between **10b** and **10a** (i.e., protons on C-17–C-19, Me-26, and Me-27) are compared, then the "MAE_{LITE}" distinction is even larger (**0.11** vs. **0.35**).

Goodman (DP4) Analysis

The MAE parameter that was employed in the above analysis is a straightforward and common method for judging the relative "goodness of fit" between computed and experimental NMR data sets.[vii] Smith and Goodman recently introduced a more sophisticated, statistical analysis–namely, the DP4 probability,[viii] which was developed for the specific instance where a single experimental NMR spectrum could be assigned to one of many possible diastereomeric candidate structures. Given the relative frequency with which this dilemma is encountered in natural product structure elucidation, it is perhaps no surprise that the DP4 probability has already found use in this arena.[ix] The DP4 protocol[viii] specifies use of the MMFF force field to optimize conformer geometries, which are then subjected to single-point DFT NMR calculations (with the B3LYP functional). The Boltzmann weighted chemical shifts thus generated are then used to calculate the DP4 probability for each candidate structure. This final step is aided by use of an online applet.[x] This analysis was applied to each of **10a** and **10b** vs. δ_{EXP}. The DP4 probabilities also indicate that the truncated structure **10b** (i.e., the "diaporthichalasin-like" stereoisomer) is a much better fit than **10a** with the spectroscopic data reported for phomopsichalasin with a >99.5% confidence interval (Table 1).

The above analyses were based on NMR data recorded in MeOH. We felt we would gain additional confidence in our conclusions if we could analyze the experimental NMR data for diaporthichalasin (**7**) in the more common solvent $CDCl_3$, with which we have considerably more experience in comparing computed with experimental proton chemical shift data sets. Professor Pornpakakul kindly provided to us a generous sample of diaporthichalasin (**7**). We recorded and interpreted a set of 1D and 2D NMR spectra in $CDCl_3$ (see Table S1).

We repeated the DFT computational analysis for **10a** and **10b** using PCM $CHCl_3$ solvation. Comparison of these computed δ_{DFT} values with our $CDCl_3$ data across the same subset of protons as before (cf. Table 1) resulted in MAE_{FULL} values of **0.25** vs. **0.10**, MAE_{LITE} values of **0.34** vs. **0.09**, and DP4 probabilities of <0.5% and >99.5% (Table S6). Again, the matches with the computed values for the "diaporthichalasin-like" substructure **10b** are far better. In addition, the magnitudes of the MAE values for the MeOH vs. the $CHCl_3$ analyses are strikingly similar, not only lending confidence that this methodology is applicable to a polar protic solvent, but also further (and strongly) implicating that the phomopsichalasin sample from the 1995 work has structure **7**.

A final set of experimental data is most compelling. The published NMR data on which the analyses were performed that led to the assignments of **1** and **7** were recorded in two different solvents (methanol-d_4 and DMSO-d_6, respectively). We therefore also recorded spectra for the diaporthicahalasin (**7**) sample in each of these two solvents (Tables S2 and S3). As we now suspected would be the case, the data for **7** in methanol-d_4 were essentially identical to those reported for the sample named phomopsichalasin (and incorrectly assigned structure **1**) in 1995. Thus, it is clear that phomopsichalasin and diaporthichalasin are the same compound, both having structure **7**. One important ramification is that the hypothesis of a spontaneous cyclization of **4** to give phomopsichalasin/diaporthichalasin (**7**) is still viable. Accordingly, we are continuing our efforts to synthesize polyene **4** in order to study its inherent IMDA reactivity.[xi]

Insights from the Initial Analysis of NMR Data of Phomopsichalasin

It is instructive to consider the analysis and logic that led to the incorrect assignment of the relative configuration of phomopsichalasin as **1**.[i] The ^{1}H NMR spectrum for phomopsichalasin was reported in methanol-d_4 (and MeCN-d_3 in order to obtain a few additional J values). Three methylene or methine proton resonances (H-19$_{eq}$, H-20$_{eq}$, H-18)

were obscured by allylic methyl group resonances. In retrospect, dispersion of these resonances, in particular that of H-18, would have been helpful since H-18 is the methine proton at the stereogenic center whose relative configuration we now know to be incorrect. To reveal those overlapped resonances and extract essential coupling constants, we performed complementary experiments by i) recording the spectrum of **7** in methanol-d_4 at a higher field strength (850 MHz) or ii) performing an NMR titration with benzene-d_6 (into methanol-d_4). In the former, we could decipher all of the J-values of H-18 (ddddq; J = 4.1, 4,1 12.3, 12.3, and 6.5 Hz; Figure S2). This full coupling constant analysis was integral to "walking" the coupled protons around the A-ring of the *trans*-fused decalin system and to assigning the orientation of H-18 to be axial rather than equatorial (e.g., the H-18 resonance includes two trans-diaxial vicinal coupling constants).

Another uncertainty was raised by the peculiar report of a doublet (δ = 0.78) with coupling constant of 2.2 Hz for Me-27. This incorrect assignment resulted from overlapped resonances for Me-11, Me-26 and Me-27 at ca. 0.8 ppm. This was clarified as well by a benzene-d_6 titration (Figure 2, Panel A), which cleanly resolved all three methyl groups and allowed us to observe the coupling constant for the doublet for Me-27 (J = 6.5 Hz), which was consistent with the J value in the multiplet assigned to H-18. However, in retrospect, even simpler solutions to this problem were available: i) by straightforward line-broadening (resolution enhancement) analysis of the ^{1}H NMR data for the three methyl resonances partially overlapped at ca. 0.8 ppm in methanol-d_4 (d for Me-11, s for Me-26, and d for Me-27; Figure 2, Panel B), we could deduce directly the correct coupling values for each of the doublets; ii) upon recording the spectrum of **7** in CDCl$_3$ (Table S1) we observed that the resonance for Me-11 (assignment confirmed by COSY) was well resolved from others, allowing the 6.5 Hz J value to be easily observed.

Finally, an unusual NOE was reported between Me-27 and H-4 in the methanol-d_4 spectrum of phomopsichalasin.[i] Spatial proximity between these two protons appears to be inconsistent with either structure **1** or structure **7**. Again, this misinterpretation of the (NOESY) NMR data can be attributed to the three overlapping methyl resonances at ca. 0.8 ppm (cf. Figure 2), which introduces uncertainty in the interpretation of the NOESY correlation peak. In fact, an NOE between Me-11 and H-4 was reported in the Pornpakakul studies,[ii] and that interaction is clearly consistent with **7** (and highly unlikely for structure **1**).

Conclusion

Detailed NMR spectroscopic analysis and pertinent computational models were used i) to show that the structure originally proposed for phomopsichalasin (i.e., **1**)[i] was incorrect and ii) to generate a hypothesis for an alternative structure assignment. Specifcially, we deduced that the structure of phomopsichalasin is, in fact, identical to that of the more recently reported diaporthichalasin (**7**).[ii] To validate our analyses, we then showed that the proton and carbon NMR spectra of an authentic sample of diaporthichalasin recorded in methanol-d_4 were in excellent agreement with the NMR data reported for phomopsichalasin.[i]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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(xi). We have also seen evidence for dehydrative and subsequent oxidative degradation events in samples of **7** upon prolonged storage in a solution of CDCl$_3$. More specifically, ^{1}H NMR resonances for the aromatic and benzylic protons in **7** diminished, and an intermediate species we suggest to be the enamide **11** was observed by LCMS and ^{1}H NMR spectroscopy (CDCl$_3$, new doublets at δ 7.06 and 6.81 for aromatic resonances and new singlets at δ 5.57, 5.42, and 5.35, which are consistent with H-10, H-15, and H-7 in **11**, respectively). Over additional time, resonances for the oxidative cleavage product, 4-hydroxybenzaldehyde (**12**), were observed (and confirmed by a doping experiment), and LCMS evidence for a new species having the mass of succinimide **13** was seen. Efficient aerobic cleavage of related enamides has been previously observed. For example, Banfi L, Andrea B, Francesco C, Giuseppe G, Naz F, Riva R, Zitoa P. Synlett. 2010:85–88. Gunawan S, Nichol GS, Chappeta S, Dietrich J, Hulme C. Tetrahedron Lett. 2010; 51:4689–4692. [PubMed: 20802841]
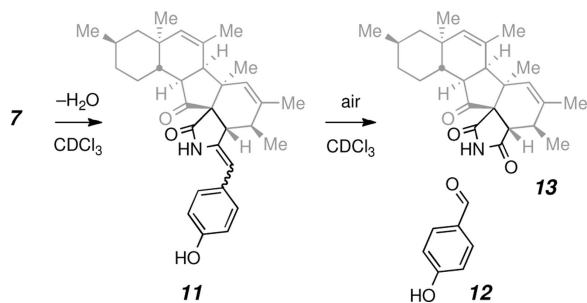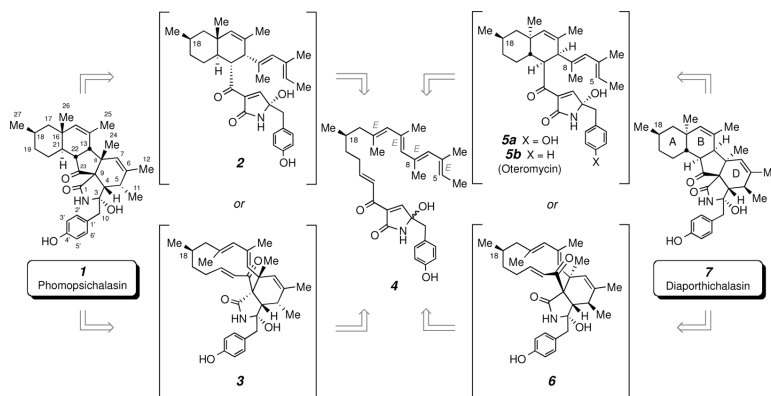
Scheme 1.
Biosynthetic consideration of the possible origin of structures **1** and **7**, assigned to phomopsichalsin and diaporthichalasin, respectively, point to the common intermediate hexaene **4**. For comparison purposes, the absolute configuration of C-18 is held constant in all structures. The absolute configuration of natural phomopsichalasin/diaporthichalasin (**7**) is unknown.

A 'Grant numbers'

8eq

8ax

δ = 1.19

H_ax

H_eq δ = 1.68

B

9a (cf. 1)

9b
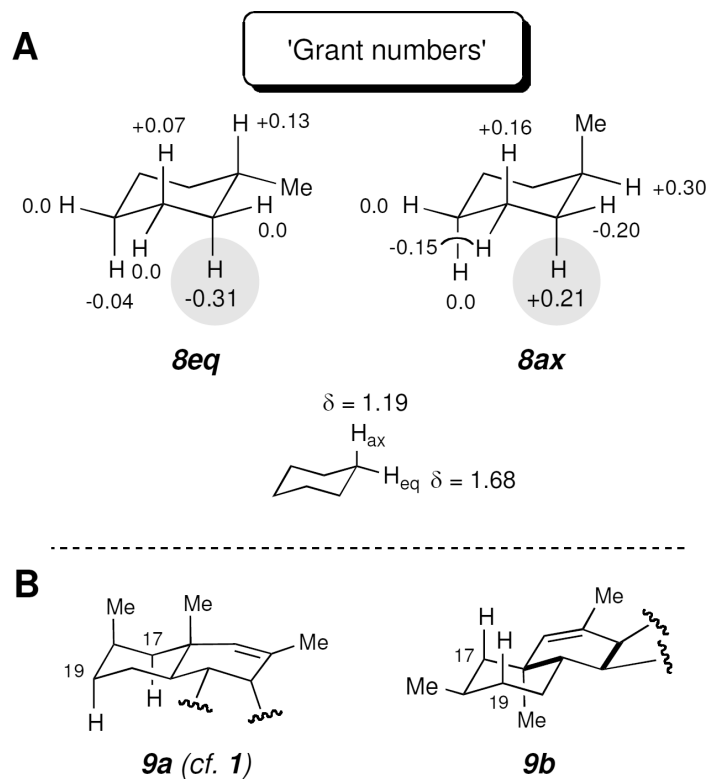
Figure 1.
Panel **A**. "Grant numbers:" The incremental chemical shift perturbations imposed by a methyl substituent in the equatorial (cf. **8eq**) vs. the axial (cf. **8ax**) position of a chair-like cyclohexane.[iii] Positive values refer to downfield (and negative upfield) changes in the chemical shift of the axial or equatorial protons of cyclohexane itself. Panel **B**. *trans*-Fused octalin subunit of structure **1** (**9a**) vs. its equatorial 18-Me counterpart (**9b**).
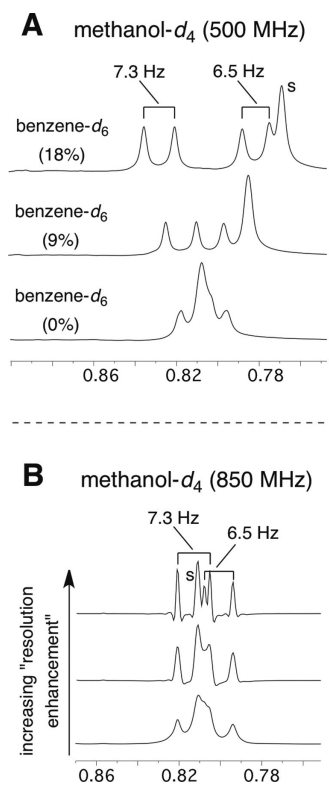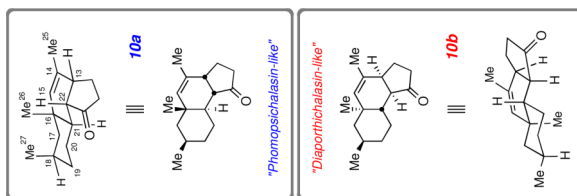
Figure 2.
Insets of methyl regions of ^{1}H NMR spectra of **7** in various solvents. Panel A: in methanol-d_4 containing varying percentages of added benzene-d_6 in order verify the presence of two methyl doublets (with J values of 7.3 and 6.5 Hz). Panel B: in methanol-d_4 with increasing levels of "resolution enhancement," which confirmed these J values.

Table 1

Comparison of the Computed Proton NMR Chemical Shifts (Δ_{dft}) for Structures **10a** and **10b** with the Experimentally Observed Shifts (δ_{exp}) for the Analogous Subset of Nuclei in the Spectrum Reported for Phomopsichalasin ("1") in Methanol-d_4.

	δ_{DFT}[a] (MeOH)		δ_{EXP}	$|\Delta\delta|$ for δ_{DFT}[a] vs. δ_{EXP}	
ATOM #	**10a**	**10b**	**"1"**[b]	**10a** vs. **"1"**[b]	**10b** vs. **"1"**[b]
13	2.68	2.70	2.85	0.17	0.15
15	5.49	5.42	5.36	0.13	0.06
17$_{\text{eq}}$	1.41	1.40	1.45	0.04	0.05
17$_{\text{ax}}$	1.42	0.92	0.67	0.75	0.25
18	1.78	1.65	1.62	0.16	0.03
19$_{\text{eq}}$	1.38	1.58	1.64	0.26	0.06
19$_{\text{ax}}$	1.36	0.81	0.56	0.80	0.25
20$_{\text{eq}}$	1.45	1.55	1.50	0.05	0.05
20$_{\text{ax}}$	1.45	1.32	1.10	0.35	0.22
21	1.55	1.49	1.42	0.13	0.07
22	2.34	2.34	2.24	0.10	0.10
25	1.73	1.75	1.88	0.15	0.13
26	0.99	0.90	0.80	0.19	0.10
27	1.05	0.81	0.78	0.27	0.03
		MAE$_{\text{FULL}}$[c] =		0.25	0.11
		MAE$_{\text{LITE}}$[d] =		0.35	0.11
		DP4 probability[e] =		<0.5%	>99.5%

[a] Values for δ_{DFT} were determined by referencing the computed isotropic chemical shifts (σCOMP) for all nuclei to the isotropic chemical shift for the protons of TMS (σREF), both of which were computed at the IEFPCM-WP04/pcS-2//M06-2X/6-31+G(d,p) level of theory.

[b] "1" = "the spectroscopic data reported for phomopsichalasin."

[c] Mean absolute error ($|\Delta\delta_{\text{AVE}}|$). Calculation includes all proton nuclei that are listed in the table.

[d]Including only the subset of proton nuclei 17–19, 26, and 27.

[e]Scaled δDFT values derived from chemical shifts computed at the B3LYP/6-31G(d,p)//MMFF level of theory were employed (see ref. viii and the Supporting Information for details).