The Discreet Structural Diversity of Briarellins: DU8+ Guided Multiple Structure Revisions Yielded Two Unknown Structural Types.

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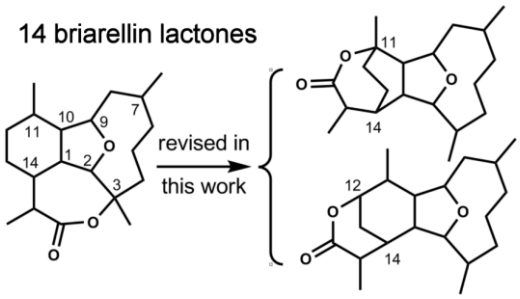
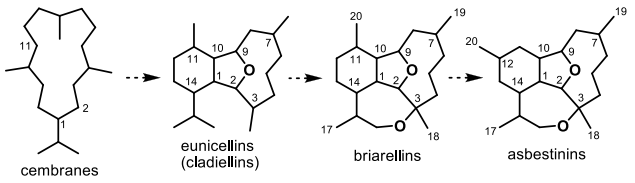


ABSTRACT: Briarellins, a subset of C2-C11 cyclized cembranoids, were proposed to contain a C3-C14 ether or lactone bridge, similar to asbestinins. However, the total synthesis of the proposed structure of briarellin J revealed a misassignment. We revisited briarellins, computationally, with the help of a recently developed hybrid DFT/parametric method, DU8+, and revised the structures of briarellin C14-C3 ε-lactones to new structural types containing either a C14-C11 or C14-C12 lactone bridge. The original structures of briarellin and asbestinin ethers were confirmed.

Briarellins, a subset of C2-C11 cyclized cembranoids were first isolated in 1995[1] from gorgonian octocorals and soft corals, the same species that produced a related structural type, asbestinins, a decade earlier, Figure 1.[2,3] The initial isolation of briarellins A–D was in quick succession followed by the discovery of briarellins E–I,[4] then briarellins J–P,[5] briarellins Q–R,[6] and finally briarellins S,[7] and T,[8] bringing the total to twenty. Several asbestinins and briarellins exhibited useful pharmacologic properties, generating broad interest in the synthetic community, with successful syntheses of two of these diterpenes by Overman, confirming the structure of briarellins containing the ethereal C3-C14 link, i.e. briarellins E and F.[9] The original discovery and structure assignment of asbestinins was facilitated by the availability of several X-ray structures. In contrast, the structures of briarellins were elucidated mainly by NMR.



Synthesis of the proposed structure of briarellin J by Crimmins revealed the structural misassignment.[10] This uncertainty with the briarellin structure persisted for a decade.

With the help of the recently developed hybrid DFT/parametric computational method, *DU8+*,[11] we analyzed the NMR data for the published briarellins and now report that the majority of them require structure revision. Figure 2 summarizes the validated and revised structural types in the briarellin and asbestinin series of diterpenes.

Figure 1. Biosynthetic path from cembranes to briarellins and asbestinins.

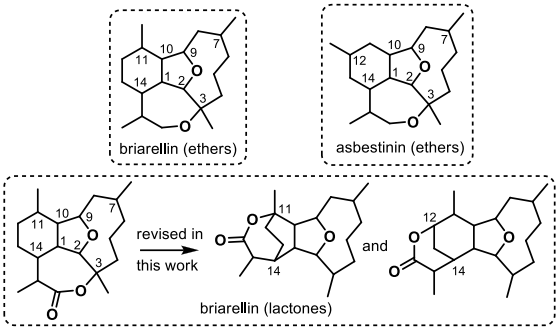


Figure 2. Correct originally proposed structural types of briarellin and asbestinin *ethers* (top), and the revised structural types of briarellin *lactones* (bottom).

The major challenge of using NMR-based computational methods for validation or revision of briarellin/asbestinin structures is size and conformational flexibility. These diterpenes contain a minimum of 20 carbons and are often functionalized with long-chain acyl groups. Evaluation of large ensembles of conformers for these molecules require considerable computational resources. As *DU8+* utilizes light DFT theory levels for both the candidate structure optimizations and calculations of NMR parameters, it became feasible to evaluate these diterpenes in a relatively short time – less than 2 hours of wall-clock time per conformer on a 16 core compute node of a Linux cluster – validating or revising their structures as necessary.[12] *DU8+* performed well in the past as exemplified by numerous reassignments of complex natural products (NP), including halogenated marine NPs,[11] triquinanes[13a], oxirane-[13b] and oxetane-[13c] containing NPs, oxo-bridged anti-Bredt natural products,[14] etc. With recent parametric corrections developed expressly for a DFT GIAO approach in chloroform (PCM),[15] the current accuracy of the ^{13}C chemical shifts computations with *DU8+* is better than 1.1 ppm root-mean-square deviation (rmsd). This allows for a much more confident differentiation of diastereomers possessing very similar NMR spectra, enhancing the utility of the method.

DU8+ analysis of all reported briarellins suggested that the structures containing the C3-C14 ethyleneoxy (i.e. ethereal) bridge, such as briarellins E, F, I, S, and T were assigned correctly, while the C3-C14 bridged *lactones*, briarellins A–D, H, and J–R were misassigned. Our analysis of briarellin J revealed two most severe deviations of the calculated and experimental ^{13}C NMR data: the chemical shift for Me17 was calculated at 11.1 ppm, which deviated by more than 6 ppm from the experimental value of 17.7 ppm. Similarly, the experimental value for C13 (16.8 ppm) deviated by almost 7 ppm from the calculated value of 23.7 ppm. In fact, the calculated ^{13}C chemical shift values for all CH_2 carbons in briarellin J were found to be greater than 23 ppm, making the experimental CH_2 signal at 16.8 ppm incompatible with the proposed structure.

The presence of multiple overlapping signals and insufficient scalar coupling data in the proton spectrum of the experimental data made the re-assignment process challenging. However, after trial and error, a revision which matched the ^{13}C NMR experimental data was identified. This revision required a lactone bridge connecting carbons C14 and C11, while the C11-acetate was moved to position C3, Figure 3. In this paper, we show the absolute stereochemistry of the core stereogenic centers C1,2,9-10,14-15 as determined by Overman in the synthesis of briarellins E and F.[9] (The 1980 Clardy's paper[2] depicted the opposite configuration of the asbestinin framework, but no absolute configuration was implied).

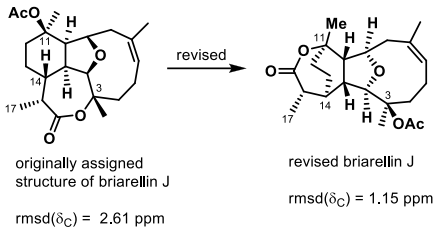


Figure 3. Original and revised briarellin J.

The calculated NMR data for the new dioxabicyclo [10.3.2.1^{3,10}.0^{2,11}]octadecane core of the revised briarellin J was closely comparable to the experimental NMR chemical shifts data, accommodating both outliers: Me17, calculated at 17.9 ppm ($\Delta\delta_{exp}$ = 0.2 ppm), and C13, calculated at 17.0 ppm ($\Delta\delta_{exp}$ = 0.2 ppm). The most recent *DU8+* ^{13}C training set gave rmsd=1.09 ppm for >7500 reliable experimental data points. The overall accuracy of the match for the revised briarellin J (rmsd = 1.06 ppm) was similar, if not slightly better than that of the *DU8+* training set, which imparts confidence in our computationally-driven revision of this rather complex and conformationally flexible diterpene.

The DFT energy of the revised briarellin J is approximately 5 kcal/mol lower than that of the originally proposed structure, possessing the C3-C14 bridge. This would suggest that the formation of briarellin *lactones* results from oxidation of a potential progenitor C16-alcohol into a carboxylic acid, followed by a transannular lactonization step to yield the thermodynamically more stable C11-C14 lactone.

Analysis of the experimental ^{13}C NMR data for briarellins and asbestinins suggested that the ^{13}C chemical shift of Me17 could serve as a criterion to differentiate between structures containing the original triatomic C3-C14 bridge and the structures possessing the new structural motif, i.e. the C11-C14 bridge. It did not escape our attention that all natural products containing an ethyleneoxy (ethereal) linker had the chemical shift for Me17 in the 10-11 ppm range, while for all the *lactones* this value fell consistently into another narrow range of 17.4-17.9 ppm, with the exception of briarellin H (19.4 ppm). The effect of the C16 carbonyl in lactones (or lack thereof in ethers) on the chemical shift of this methyl group was negligible, as demonstrated by the calculated Me17 chemical shift value of 11.1 ppm for the originally proposed structure of briarellin J. Synthesis of this putative structure of briarellin J by Crimmins established that the Me17 peak in the ^{13}C spectrum of such C3-C14 lactone was indeed found at 12 ppm.[10] From this combined experimental and computational evidence, we inferred that the high field ^{13}C chemical shift values of 10-12 ppm for Me17 could be a reliable characteristic of a C3-C14 bridge, regardless whether it belongs to a cyclic ether or ester. As shown in Figure 4, this hypothesis was correct, i.e. none of the briarellin lactones had a Me17 peak in the high field range of 11-12 ppm and, therefore, all required revision.

Guided by *DU8+* computations, we revised briarellin lactones, possessing the Me17 peak in the 17.4-17.9 ppm range, into the structures shown in Figure 4, all containing the C11-C14 bridge. In addition to connecting the cyclic ester through

carbon C11, some of the revisions involved swapping substituents at C3 and C6, for example, briarellins A and B.

Briarellin C has the same core structure as briarellin B. It was calculated with both long-chain fatty acyl groups truncated to acetyls, giving a good match with the experimental ^{13}C data. However, it is challenging to differentiate the exact locations (i.e. C4 *vs* C6) of the butanoyl *vs* octanoyl tails.

Briarellin D has the same core as briarellin K; they differ by the acetyl *vs* butanoyl substitution. Both revised structures gave a good match of the experimental and computational data, although chemical shifts of C3 and C11 needed swapping. Occasional reassignment of individual carbons with the same peak multiplicity was needed in this and other cases – see Supporting Information Section.

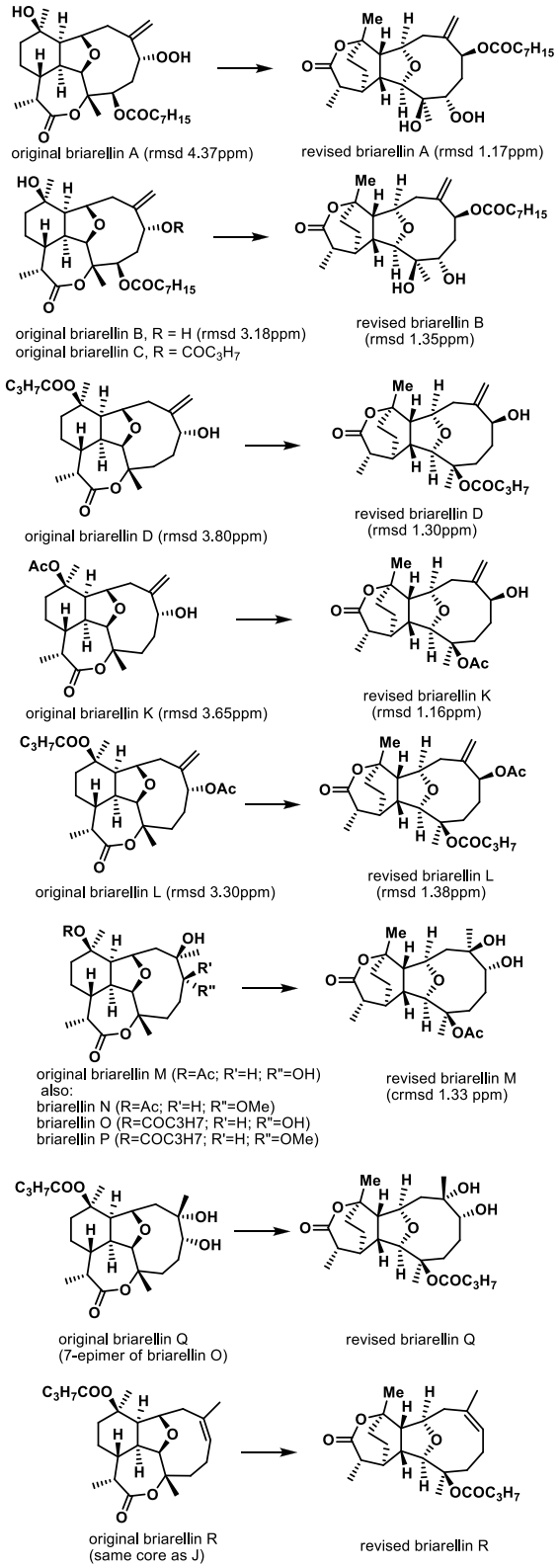


Figure 4. *DU8+* guided revisions of briarellin lactones.

In briarellins containing hydroxy group(s) in the conformationally flexible loop C4-C8, such as briarellins M and briarellin Q, the intramolecular hydrogen bonding presented significant challenges for assigning the C6-C7 stereochemistry. The main problem is that DFT computations often overestimate the stabilizing effect of intramolecular hydrogen bonds, which makes it

not ideal to use the DFT-derived Boltzmann populations in mixing the computed data from individual conformers. In this particular case, the best fit to the experimental data was produced by the diastereomers shown in Figure 4, although the rmsd values were somewhat greater than the ones obtained for briarellins lacking hydroxy groups in the flexible loop C4-C8; for example, briarellin J. This loss of accuracy of the fit for briarellins containing hydroxy group(s) at C6/C7 compelled us to assign the stereochemistry at these positions only tentatively. At the same time, there was no doubt that all the shown briarellins have the C11-C14 lactone bridge.

As noted above, briarellin H is the only member of this lactone family, which had Me17 slightly outside of the 17.4-17.9 ppm range. NMR computations for its original structure and the C11-C14 candidate structure both showed poor fit. Careful analysis of the computed and experimental data lead to the revision of its structure to yet another structural type – the C12-C14 bridged lactone, Figure 5.

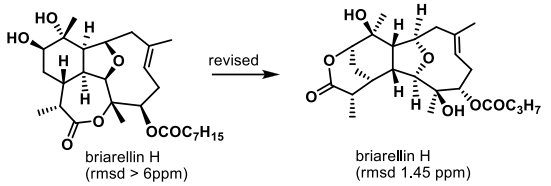


Figure 5. Revision of briarellin H.

It appears that briarellin H is the only briarellin *lactone* reported up to date, which possesses this new structural type.

The fact that the examined *ether* briarellins and asbestinins did not require revision into the new structural types does not imply that they did not require minor revisions at all. As it often happens with complex and conformationally flexible diterpenes, some asbestinins required minor revisions of the originally proposed structures, as more accurate experimental NMR data became available. For example, Rodríguez reassigned the structures of asbestinin-10, asbestinin-20, and asbestinin-21 in a revision, which involved 'relocation' of the substituents from C-4 to C6, while not requiring changes to their core framework.[6] The Clark group recently published total synthesis of several asbestinins,[16] confirming these prior revisions and further correcting the C-7 stereoconfiguration in asbestinin-21. As the difference between the two C-7 epimers is subtle, we used this opportunity to test whether *DU8+* could differentiate between these two epimers of asbestinin-21.

As Figure 6 illustrates, the first revision brought the rmsd to an acceptable value of 1.44 ppm. However, the second revision (i.e. to the C-7 epimer) produced a much better fit, rmsd = 0.81 ppm. Most importantly, the deviation of the calculated ^{13}C chemical shift value for Me19 from its experimental value exceeded 4.6 ppm in the first revision. After the second revision, this difference was reduced to ~1 ppm, confirming Clark's revision and providing another instructive example of the method's performance.

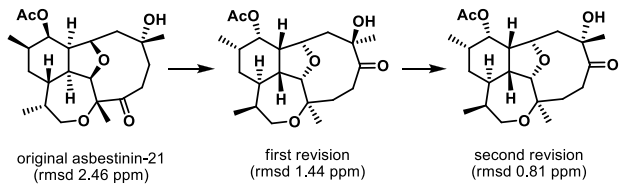


Figure 6. Two revisions of the original structure of asbestinin-21 revisited computationally.

In conclusion, briarellins A–D, H and J–R were revised to two new structural types, possessing the C11-C14 or C12-C14 lactone bridge. We have a reason to believe that these structural types are more common and not limited to the briarellin lactones revised in this study. Work continues to identify natural products containing these new structural cores.

The *in silico* revision of multiple structures of these complex and conformationally flexible diterpenes constitutes a new benchmark for the hybrid parametric/DFT method, *DU8+*, suggesting a whole new level of maturity of computational methods to aid in the structure elucidation of complex natural products. As the optimization of parametric corrections to the DFT-computed chemical shifts and corrections to the nuclear spin-coupling constants depends on the analysis of the existing massive experimental NMR datasets, the method is systematically improvable and is amenable to machine learning.

On a related note: as the quality of the experimental data reporting is of paramount importance, we welcome the appeal by the ACS Editors[17] for submission of the primary research data (FAIR Data) and the development of a new tool for sharing of primary research data, the ACS Research Data Center.[18] Depository of raw NMR data was long advocated by some of us.[19] Hopefully, the development of new tools to store and share the original experimental data will help improve the quality and reliability of scientific reporting, and further facilitate structure elucidation and stereochemistry assignment of natural products and other complex organic structures.

ASSOCIATED CONTENT

Supporting Information. Computational details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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