Addressing the Challenges of Structure Elucidation in Natural Products Possessing the Oxirane Moiety

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ABSTRACT: NMR data for natural products containing the epoxy moiety have been revisited and reanalyzed with the help of a recently developed parametric/DFT hybrid computational method, DU8+. More than 20 structures needed revision, which points to challenges in NMR solution structure assignment for molecules possessing this structural feature. Among the revised structures are achicretin 2, acremine P, aromaticane I, artanomalide B, botryosphaerhydrol, chloroklotzchin, crithmifolide, crotodichogamoin A, emervaridone C, 9α,15-epoxyafricanane, fischambiguine B, grandilobalide B, guaianolide A, guatterfriesols A and B, juncenolide G, roscotane D, secoafricane 7, taccalonolides AJ and AF, and related compounds.

INTRODUCTION

Large numbers of oxygenated natural products possess an epoxy moiety which, judging by a number of misassigned structures, presents an additional challenge for structure elucidation. As pyramidalization of carbons in epoxides is not pronounced, their protons exhibit overlapping ranges for the elucidation. As pyramidalization of carbons in epoxides is not pronounced, their protons exhibit overlapping ranges for the elucidation. As pyramidalization of carbons in epoxides is not pronounced, their protons exhibit overlapping ranges for the elucidation. As pyramidalization of carbons in epoxides is not pronounced, their protons exhibit overlapping ranges for the elucidation. As pyramidalization of carbons in epoxides is not pronounced, their protons exhibit overlapping ranges for the elucidation. As pyramidalization of carbons in epoxides is not pronounced, their protons exhibit overlapping ranges for the elucidation. As pyramidalization of carbons in epoxides is not pronounced, their protons exhibit overlapping ranges for the elucidation. As pyramidalization of carbons in epoxides is not pronounced, their protons exhibit overlapping ranges for the elucidation. As pyramidalization of carbons in epoxides is not pronounced, their protons exhibit overlapping ranges for the elucidation. As pyramidalization of carbons in epoxides is not pronounced, their protons exhibit overlapping ranges for the elucidation. As pyramidalization of carbons in epoxides is not pronounced, their protons exhibit overlapping ranges for the elucidation. As pyramidalization of carbons in epoxides is not pronounced, their protons exhibit overlapping ranges for the elucidation. As pyramidalization of carbons in epoxides is not pronounced, their protons exhibit overlapping ranges for the elucidation. As pyramidalization of carbons in epoxides is not pronounced, their protons exhibit overlapping ranges for the elucidation. As pyramidalization of carbons in epoxides is not pronounced, their protons exhibit overlapping ranges for the elucidation. As pyramidalization of carbons in epoxides is not pronounced, their protons exhibit overlapping ranges for the elucidation. As pyramidalization of carbons in epoxides is not pronounced, their protons exhibit overlapping ranges for the elucidation.

Structure revision of caespitenone is a representative example of these challenges. Initially, it was isolated from the liverwort Porella caespitans and assigned structure A. Additional 2D NMR experiments revealed discrepancies, and the structure of caespitenone was revised to an africane type sesquiterpene B. Instructively, in order to relate the mutual orientation of the two small rings in B, the authors subjected caespitenone to the Miyashita reaction conditions, with oxirane ring-opening and subsequent NOESY experiment revealing the cross-peak between the newly formed α-OH and H11 protons and thus confirming the syn orientation of the small rings in B.

We note that modern developments in computational methods for prediction of NMR spectra make the task of structure elucidation less strenuous. For example, our own hybrid DFT-parametric DU8+ computations on the revised structure B took only 11 min of total computational time ("wall-clock time") on a single node of a Linux cluster and gave excellent agreement with both 1H and 13C NMR experimental data for caespitenone: rmsd(δH) = 1.18 ppm, rmsd(δC) > 5 ppm.

Examples of "difficult" epoxides are plentiful and include the iconic saga of hexacyclinol, for which a computationally driven revision of the originally misassigned structure was proposed by Rychnovsky, and later confirmed by Porco's elegant synthesis.

In complex oxygenated steroid systems such as the withanolides, a statistically significant sample of reliable structures and 13C NMR data sets have been compiled by Timmermann and others. As a result, empirical rules to predict chemical shift perturbations introduced by oxygen atoms, for example, the γ-gauche effect, have been developed and successfully utilized for structure correction. It is instructive...
that a considerable number of structures in need of such correction contained the epoxy moiety.

Faster computational approaches to aid NMR analysis of complex organic molecules include such parametric methods as Goodman’s DP4.10 Subsequent improvements of these approaches often address challenges related to structural features poorly described by fast (i.e., low level of theory) methods. For example, Sarotti’s improved DP+ method17 was developed to include more sophisticated calculations of chemical shifts for unusually hybridized carbons, especially in epoxides. This was recently successfully tested on a set of 24 selected spiro or terminal epoxides.12 A further expansion of DP4 probability theory, DiCE (Diastereomeric in silico Chiral Elucidation), was recently developed for higher accuracy and relatively low computational costs.

Another example of a fast parametric approach is computer-aided structure elucidation (CASE) which utilizes linear scaling of chemical shifts, offering practical tools for structure discovery.18 Again, these methods are often only as good as the quality of calculated chemical shifts. For this reason, better hybrid CASE approaches are suggested, with the DFT-computed shifts augmenting empirical corrections.15

Nuclear spin–spin coupling constants (SSCCs) contain more structural information but are challenging to compute. However, practical parametric methods are now emerging for fast and accurate evaluation of SSCCs: Bally and Rablen’s work on linear scaling of the easily computed Fermi contacts16 and our own related method which we termed relativistic force field (rff).17,18

To streamline the solution structure elucidation and validation process, we combined the rff computations of SSCCs with empirical corrections of the DFT-computed chemical shifts in an integrated DU8+ method,5 which allows for a high-throughput analysis of reported structures based on ubiquitous 1D NMR data.

DU8+ combines computations of structure and NMR properties of organic molecules at a light level of DFT theory and is implemented with the following components: (a) structure optimization: B3LYP/6-31G(d); (b) magnetic shielding: oB97X-D/6-31G(d); (c) Fermi contacts: B3LYP/ DU8; (d) scaling of the computed Fermi contacts according to refs 17b and c, using “rff” to obtain spin–spin coupling constants; (e) scaling of isotropic magnetic shielding values according to ref 5 to obtain chemical shifts.

DU8+ has expedited not only identification of misassigned structures but also accelerated the process of structure revision. The computed spin–spin coupling constants offer more intuitive guidance for generating hypothetical structure candidates than the calculated chemical shifts alone.

In the past, as a result of this high-throughput screening, we encountered a number of incorrectly assigned epoxides in the series of halogenated marine natural products (NP); for example, 1β-bromo-4α,5α-epoxyselinine19 or, in the triiquinane series, hirsutenol E.20 This prompted us to revisit NMR data for a number of NPs possessing the oxirane moiety. More than 20 of these structures needed revision.

## RESULTS AND DISCUSSION

The structures of the epoxy-containing natural products were preoptimized with the force field MMFF94 as implemented in OpenBabel.21 For structures with freely rotatable groups, conformers were generated using OpenBabel’s confab, whereas the conformers resulting from conformational changes in cyclic cores were generated manually, using Chem3D. As the empirical corrections for DU8+ were developed on a training set of 1H and 13C NMR spectra recorded in CDCl3, we use fast gas phase computations for such cases. For experimental data obtained in DMSO-d6 or methanol-d4, a PCM model with additional linear scaling of chemical shifts was utilized.22

DU8+ performs adequately in predicting spectra of complex epoxidized natural products, as exemplified in Figure 2. For

![Figure 2. Selected test cases demonstrating the accuracy of DU8+ calculations for the correctly assigned complex epoxides.](image-url)
chemical shifts for ledene epoxide 3 gave an inferior rmsd(δC) of 2.5 ppm. A similar poor match, rmsd(δC) = 2.1 ppm, was obtained for the experimental data of ledene epoxide 2, fitted with the computed data for ledene epoxide 3.

Overall, the current training set of 13C chemical shifts for DU8+ exceeds 6080 reliable experimental measurements calculated with the rmsd(δC) of 1.28 ppm. This accuracy is currently better than any method we know, especially given the fact that the set contains carbons bearing chlorine, bromine, and other heavy atoms. As a result, the majority of validated structures fall into the rmsd range of 1.0–1.8 ppm or even better. Proton spin–spin coupling constants (SSCCs) are computed with an accuracy of 0.3 Hz, as determined on the training set of more than 4K reliable experimental values. In any given case, the calculated values may deviate from the experimental values for extraneous reasons. For example, there are very few practitioners in the field reporting J-coupling constants with sufficiently high accuracy, which potentially could be achieved with approaches such as HiFSA (1H iterative Full Spin Analysis). However, as described below, most of the time the validated original (or revised) structure gives superior match across the primary two criteria, i.e., rmsd(δC) and rmsd(JHH), augmented by the secondary criterion—rmsd(δH)—which is less reliable but still useful in the final analysis.

Before we proceed any further with the examination of misassigned structures of complex natural products containing the oxirane moiety, we reiterate that stereochemistry assignment is challenging even for very small epoxidized molecules. For example, DU8+ analysis reveals that the syn stereochemistry of the reported toluene dioxide, Figure 3 (compound 11e in ref 31) is erroneous and needs revision to anti, which may further necessitate revisions in the mechanism of its formation proposed by the authors.

Figure 3. Revision of the toluene-based syn-diepoxide 11e to anti.

Africane-type sesquiterpenoid (compound 7 in ref 32) represents the most common challenge in the assignment of oxirane stereochemistry because it lacks protons at positions 4 and 5. Additionally, the two small cycles—oxirane and cyclopropane—which could be either syn- or anti- to each other, are too distant to cause significant perturbation of chemical shifts of the intervening atoms or spin–spin coupling constants to make the stereochemical assignment with confidence. DU8+ calculations on the original (syn) structure produced a relatively poor match, rmsd(δC) = 2.66 ppm, rmsd(δH) = 0.37 ppm, and rmsd(JHH) = 0.32 Hz, whereas the anti-isomer gave a much better fit, rmsd(δC) = 1.03 ppm, rmsd(δH) = 0.16 ppm, and rmsd(JHH) = 0.30 Hz. Therefore, we revise its structure to the anti-diastereomer, i.e., epi-4,5, shown in Figure 4 (relative stereochemistry is implied in this and subsequent revisions).

Figure 4. Revision of secoafricane compound 7, grandilobalide B, and crithmifolide.

The structure of grandilobalide B, Figure 4, was possibly misassigned because of similar challenges, i.e., the lack of oxirane protons and too subtle a structural perturbation introduced by the oxirane oxygen, when placed on either face of the molecule. Nonetheless, DU8+ is capable of differentiating the two stereoisomers: rmsd(δC) = 0.99 ppm for the revised, i.e., epi-5,6 structure, while the original structure gave rmsd(δC) = 1.98 ppm. The most offending chemical shifts in the original structure belonged to oxirane’s C5 (Δδ = 4.96 ppm discrepancy between the calculated and experimental values) and C15 (Δδ = 4.24 ppm). The chemical shift belonging to the lactone’s carbonyl carbon (C15) is clearly perturbed via a through-space interaction with oxirane’s lone pair, as the distance between the oxirane oxygen and the carbonyl’s carbon in the revised structure is less than 2.7 Å.

Crithmifolide, a sesquiterpene lactone from Achillea crithmifolia required a similar revision, despite the fact that one of the oxirane carbons, C3, conveniently carries a hydrogen atom vicinal to H−C2. The vicinal H−C2−C3−H constant is too small to be helpful. Nonetheless, on the basis of DU8+ analysis of chemical shifts, the structure of crithmifolide is now revised to the shown epi-3,4 diastereomer. Another candidate structure, the epi-2,3,4 isomer, gave a less accurate fit, rmsd(δC) = 1.61 ppm, rmsd(δH) = 0.16 ppm, and rmsd(JHH) = 0.23 Hz (see the Supporting Information), and was rejected.

Achicretin 2 (also referred to as sesquitunin 2) was recently isolated from Achillea cretica growing in Tunisia. It had the same molecular formula as another epichlorohydrin, guaianolide A, isolated by the authors earlier from the same plant.
resulting in the structure assignment shown in Figure 5. DU8+ computations on both originally proposed structures of achicretin 2 and guaianolide A showed irreconcilable differences with the experimental NMR data. After careful analysis of all of the discrepancies in the achicretin 2 data, another epichlorohydrin (with the chlorine atom transposed from C1 to C3) was proposed as a revision, which exhibited an excellent match with experimental data.

The only remaining inconsistency was that the original experimental data reported the H2 → C1 HMBC cross-peak and no such cross-peak was reported for H3 → C1, whereas we expected the opposite based on the computed C–H spin–spin coupling constants, J(H2–C1) < 0.4 Hz, J(H3–C1) = 8.5 Hz. Luckily, a literature search revealed that the revised structure of achicretin 2 belongs to a known sesquiterpene isolated from Achillea clavennae, i.e., 3α-chloro-4β,10α-dihydroxy-1β,2β-epoxy-5α,7αH-guai-11(13)-en-12,6α-olide.35 Besides the fact that its experimental 1H and 13C chemical shifts and proton spin–spin coupling constants, J(H2–C1) < 0.4 Hz, J(H3–C1) = 8.5 Hz, matched achicretin 2 perfectly, the authors reported only the H3 → C1 HMBC cross-peak, which is in agreement with our J(CH) computations.

We also found a report on another similar epoxide-chlorohydrin, chloroklotzchin, isolated from Artemisia klotschiana in 1985.36 According to our calculations, chloroklotzchin was also misassigned. Because its NMR data are reported in DMSO-d6, they are somewhat different from that of achicretin 2. However, we found enough similarity to suggest that chloroklotzchin is most likely the same 3α-chloro-4β,10α-dihydroxy-1β,2β-epoxy-5α,7αH-guai-11(13)-en-12,6α-olide shown in Figure 5 (see Table S1 for a comparison of the experimental data).

Guaianolide A was then revised to the 2-chloro-3,4-epoxy structure shown in Figure 5. The revised structure of guaianolide A is depicted keeping the absolute configuration at the C6–C7 ring fusion the same as in 3α-chloro-4β,10α-dihydroxy-1β,2β-epoxy-5α,7αH-guai-11(13)-en-12,6α-olide (i.e., revised achicretin 2).

The fact that achicretin 2 and guaianolide A were isolated from the same plant may point to their common precursor (Figure 6), diepoxide artecanin, which itself had an interesting history of reassignment and renaming (also known as chrysartemin B).37 As far as we can tell, the revised guaianolide A is the only example of the formal HCl opening of 1,2-epoxide (i.e., not the 3,4-epoxide) in artecanin or canin (the incorrect structure of chloroklotzchin notwithstanding). However, a related 9-acetate, algerianolide, was isolated from A. lieutista and its structure was established by X-ray analysis.38 Comparison of the experimental 13C NMR chemical shifts for guaianolide A and algerianolide reveals a strong similarity. In fact, when the carbons proximal to the C9–OAc substitution, i.e., C7–C10 and C14, were omitted from this comparison, the remaining 10 carbons gave a rmsd of 0.37 ppm, Figure 7.

Another chlorohydrin-containing sesquiterpene lactone, artanomalide B,39 was isolated from Artemisia anomala, one of the species of the genus Artemisia, broadly used in Chinese folk medicines. As shown in Figure 8, we found irreconcilable differences between the calculated and experimental NMR data for artanomalide B, prompting revision. The revised structure (the shown epi-1,2,3 diastereomer) gave an excellent agreement with the experimental NMR, except this structure was in conflict with the NOESY cross-peak between H3 and H5, reported in the paper39 and originally used to assign the C3(Cl) stereochemistry. However, our additional examination of the NOESY spectrum in the supplementary data did not
reveal any H3–H5 cross-peaks, indicating an error in interpretation of the experimental data. Thus, artanomalide B is revised to the shown 1,2,3-epimer.

By analogy with the common diepoxide precursor in Figure 6, it is plausible that the revised artanomalide B is a result of the 3,4-epoxide ring opening in the 10-epimer of artecanin, as shown in Figure 8. This diepoxide precursor is known; it was isolated from *Tanacetum parthenium* (incorrectly named *epi*-10-canin) and independently from *Ajania fastigiata* and named isochrysartemin B. According to the current consensus on the structures of canin and artecanin, isochrysartemin B should be referred to as *epi*-10-artecanin.

If epichlorohydrin sesquiterpene lactones of this type are products of an HCl-induced ring opening of one of the epoxide units in diepoxides canin/artecanin or their diastereomers, then 3β-chloro-4α,10α-dihydroxy-1β,2β-epoxy-5α,7αH-guai-11(13)-en-12,6α-olide isolated from *Achillea ligustica* in 2003 (reported as compound 6 in the original paper) should have resulted from an anti-diepoxide precursor. As such a precursor is not described in the literature, we were curious if this sesquiterpene lactone itself is misassigned. DU8+ computations did confirm this hypothesis, as the calculated data for the 9-epimer of diol 3 matched the experimental data for “epoxide 2” with rmsd(δ_c) = 1.41 ppm. Literature search revealed that the same two diol epimers were isolated from a similar soft coral, *Sinularia intacta*, and correctly characterized. Moreover, in 2011, Nakata and co-workers synthesized both pairs of 9-epimers for the diols and the epoxides, noting that the epoxide is definitely misassigned. Our computations of 13C NMR chemical shifts produced excellent matches on all four synthetic compounds, diol 3 showed satisfactory agreement with our calculated data, epoxide 2 clearly needed revision. The epoxide carbons normally exhibit higher field chemical shifts compared with alcohols. However, comparison of experimental 13C chemical shifts for the putative epoxide 2 and diol 3 revealed close similarity, rmsd(δ_c) = 1.52 ppm, indicating that “epoxide 2” could be an isomer of diol 3. DU8+ computations did confirm this hypothesis, as the calculated data for the 9-epimer of diol 3 matched the experimental data for “epoxide” 2 with rmsd(δ_c) = 1.41 ppm.

Another common challenge is that diols (or chlorohydrins) are often confused for epoxides. For example, 9α,15-epoxyafricanane 2 was isolated together with the corresponding diol, 9α,15-dihydroxyafricanane 3, from soft coral *Sinularia disjecta*, Figure 10. While the experimental NMR data for
i.e., two synthetic epoxides and two diols, Figure 10B. Possibly, because of one discrepancy with the $^{13}$C NMR data in the original isolation paper (in which C10 was assigned 29.6 ppm), Nakata and co-workers stopped short of revising the misassigned epoxide to 9$\beta$15-dihydroxyafricanane, writing in the conclusions, “Although we are uncertain about the structure of the compound isolated from the soft coral _Sinularia dissecta_, our results indicate that the structure of the natural product named epoxyafricanane was incorrectly assigned.”

With DU8+, we double-checked computationally that the purported 9$\alpha$,15-epoxyafricanane (compound 2 in ref 44) is not a chlorohydrin, i.e., a product of HCl-induced epoxide opening. This could be a plausible epoxide degradation channel, as some batches of CDCl$_3$ contain considerable amounts of HCl capable of epoxide ring-opening. The computational results clearly indicate that this is not the case, as the match with the experimental data among the potential chlorohydrins had poor rmsd($\delta_C$) > 2.1 ppm. On the other hand, removal of the offending C10/29.6 ppm peak from the experimental data set improved the fit for 9$\beta$,15-dihydroxyafricanane from a rmsd($\delta_C$) 1.50 ppm to 1.26 ppm, when we ignored the same 29.6 ppm peak ascribed to its C10 carbon in ref 44.

After all of the considerations, we revised 9$\alpha$,15-epoxyafricanane to 9$\beta$,15-dihydroxyafricanane, Figure 10A. The misreported C10 observed at the 29.6 ppm peak most likely belongs to grease or another impurity in the sample.

A second example of a hydroxylated compound confused for an epoxide is botryosphaerihydrofuran 5, isolated from the endophytic fungi _Botryosphaeria rhodina_, Figure 11. In addition to problems with underestimated $^{13}$C chemical shifts for the epoxide carbons C3a and C8a, calculations of the NMR spectra for the originally proposed structure revealed many other irreconcilable differences, including the challenge that $^{13}$C chemical shifts for carbons C6, C7, C7a, and C8 were deviating significantly from the experimental data. Eventually, we concluded that this NP possesses an extra carbonyl group, with its $^{13}$C peak being overlooked in the experimental spectrum. Another metabolite, botryosphaeridione 4, Figure 11, isolated from this fungus in the same campaign and correctly assigned, possessed an extra carbonyl group (C7) and helped resolve this reassignment impasse. We hypothesized that botryosphaerihydrofuran belongs to the eremophilane structural type. While we do not have an explanation for the reported HRMS data, there is very little doubt that the NMR spectra for botryosphaerihydrofuran belong to the revised structure shown in Figure 11.

This exact revised structure was not reported in the literature. However, our literature search yielded a very similar metabolite, microsphaeropsin, isolated by König, who investigated marine sponges _Ectyplasia perox_ and _Myxilla incrustans_ for associated fungal strains. There are only two differences: microsphaeropsin is a methyl acetal, not a hemiacetal, and it is an 11-epimer of the revised botryosphaerihydrofuran (we do not address the issues of absolute configuration in this work). DU8+ confirms the correct assignment of microsphaeropsin with high accuracy. As shown in Figure 11, the experimental $^{13}$C data for both compounds match very closely, except for the carbons proximal to C11 and C8 in microsphaeropsin, i.e., the ones most affected by the two differences in these structures. This additional experimental evidence imparts confidence that our computationally driven revision of botryosphaerihydrofuran is indeed correct.

A new diterpenoid, aromaticane I, recently isolated from a herb _Curcuma aromatica_ Salisb. possessed a peculiar epoxidized bicyclo[2.2.2]octene moiety, Figure 12. However, a cursory analysis of its $^{13}$C chemical shifts, especially C15 and C16,
indicated that the compound is unlikely to have the purported oxirane. Further computational analysis confirmed that aromaticacne I is misassigned and that the experimental data belong to the structural type of a kauroic acid. Additional searching of the literature gave a good match with the known ent-11α,16α-epoxy-15α-hydroxy-16δ-kaur-19-β-oic acid50 (see Table S4 for a comparison of experimental chemical shifts).

Furthermore, on the basis of DU8+ analysis, aromaticacne D, a 5,10-epoxy sesquiterpene (an oxetane, not an oxirane), also isolated from the same herb needed a relative stereochemistry correction, as shown in Figure 12.

DU8+ computations on the structure of crotoloflane diterpenoid crotodichogamoin A,51 recently isolated from the root of Croton dichogamus, gave a poor match with the experimental data. Its 2,4,9-epimer matched these data the best, and we revised the structure of crotodichogamoin A to its 2,4,9-epimer shown in Figure 13. We do not have a good rationale that crotodichogamoin A is isolated together with a very similar diterpene crotohaumanoxide, which was previously characterized by X-ray as syn- not anti-diepoxide.52 Figure 13. Incidentally, a control DU8+ run gave a perfect match for crotohaumanoxide, rmsd(δC) = 1.04 ppm. The stereochemistry of crotodichogamoin A51 was assigned by analogy with that of crotohaumanoxide. Nonetheless, we assert that the structure of crotodichogamoin A needs revision and that its 2,4,9-epimer is, at this point, the best structure.53

A new terpene--polyketide hybrid meroterpenoid, emervaridone C, containing an oxirane moiety derived from an exocyclic terminal alkene, Figure 14, was isolated from cultures of fungus Emericella sp. TJ29.54 Its putative alkenic precursor, emervaridone B, was characterized by X-ray. However, epoxidation of exomethylenecycloalkanes could occur from either face, and the resulting spiro-oxiranes constitute a particular challenge to structural assignment. Therefore, it is not surprising that DU8+ analysis necessitated the revision of stereochemistry of emervaridone C at carbon C3.

The largest deviation in calculated 13C chemical shifts for the originally proposed structure, ΔδC = 7.6 ppm, is observed for the oxirane’s methylene C9. It is surprising that the next largest deviation, i.e. ΔδC = 4.0 ppm, belongs to CS, accented with the second yellow circle in Figure 14. In the flat drawing, CS appears distant from the problematic spiro-oxirane: CS and the oxirane’s oxygen are five bonds apart from each other. The structure of emervaridone C is conformationally rigid, it can be described by a single low energy conformer shown in Figure 14 for the revised structure. In it, the oxirane oxygen is “intruding” into the σ* orbital of the H–C bond, with an O···H distance of only 2.22 Å, which qualifies for a nonclassical hydrogen bond, O···H–C. This produces an ~3 ppm effect on the calculated chemical shift of CS, thus providing a secondary criterion for this structure revision.

A similar rationale based on the oxirane–methylene chemical shift, augmented with a secondary criterion, is applicable to the case of the correctly assigned fungal metabolite isotrichodermin, Figure 15.55 The overall rmsd(δC) favors the correct isomer, but the differences are marginal for confident assignment. However, analysis of individual deviations reveals that the chemical shift of the oxirane’s methylene deviates from the experimental value by 2.9 ppm in the alternative incorrect structure. The additional criterion is that the calculated chemical shift for a proximal methyl group, Me14, in the incorrect candidate structure deviates by 4.3 ppm, which imparts confidence that the originally assigned structure is indeed correct.

Such a helpful secondary criterion is not always available. This constitutes a recurring challenge for stereochemical assignment of epoxidized exocyclic methylenes. For example, calculations for the correctly assigned structure of plagiochiline J, Figure 16, isolated from the liverwort Plagiochila Fruticosa,56 revealed that, with the omission of the oxirane’s methylene carbon, the correctly assigned original structure and its spiro epimer both gave a very good match, rmsd(δC) = 1.14 and 1.09 ppm, respectively. Inclusion of C11 offers only a marginal
preference of 1.19 ppm over 1.32 ppm—not a comfortable margin at all comparable to the DU8+ accuracy of 1.28 ppm. What saves the day and validates the original correct assignment for plagiochiline J is that the calculated chemical shift for C11 in the incorrect candidate structure deviates by 3.3 ppm from the experimental value, whereas it is predicted within 1.8 ppm for the correct isomer, Figure 16. This difference of only 1.5 ppm in calculated values underscores the importance of accurate methods for computing NMR chemical shifts (or SSCCs) with a narrow distribution of errors.

A complex hapalindole-related alkaloid fischambiguine B also possesses a spiro-oxirane moiety. Its DU8+ analysis seems to confirm that the discrepancy in computed and experimental 13C chemical shift for oxirane’s methylene carbon (C26) is indeed a reliable criterion for validating spiro-oxiranes. As shown in Figure 17, the stereochemistry at the spiro carbon C25 of fischambiguine B needs revision, as the 13C chemical shift value for C26 in the originally assigned configuration deviates by 7.9 ppm. The spectra were acquired in DMSO-δ6, known to complicate proton chemical shift computations of alcohols. This complication is due to the difficulty in predicting coordination of solvent through H-bonding with OH or NH moieties and the relative strength of the intramolecular hydrogen bonds. The proton chemical shifts are therefore omitted from the analysis, as we normally do not consider their calculated values in DMSO reliable, especially for the substrates capable of forming hydrogen bonds with the solvent.

A potential problem with the revised structure is that the oxirane’s methylene exhibits a NOESY cross-peak with the C(10)−OH group, implying that the CH2 and OH groups are syn, i.e., on the same face of the molecule. However, analysis of the calculated structures shows that, due to insignificant pyramidalization of the oxirane’s methylene, the distance between C(10) and the proximal H26 atom of the methylene group is nearly identical in both structures, ~4.2 Å. If one assumes free rotation of the OH group, these two structures should be indistinguishable on the basis of this NOESY cross-peak. As further shown in Figure 17, the difference is that the revised structure has a strong hydrogen bond between the OH group and the oxirane oxygen. This rotates the OH group favorably and reduces the distance between H26 and OH to 3.8 Å. In the original structure, such an H-bond is impossible and, in the lowest energy conformer, OH points in the direction of indole’s π density, with the OH−H26 distance increasing to 4.85 Å. The same conformation is expected with an H-bonded solvent molecule.

Guatterfriesols A and B58 were recently isolated from the stem bark of G. friesiana and assigned the sesquiterpene lactone structures shown in Figure 18. Our computations revealed that the experimental data for guatterfriesol A fit the structure originally proposed for guatterfriesol B. Guatterfriesol B, in turn, is revised to its 4,5,6-epimer. This revision could accommodate a hypothesis of a common alkenone precursor, epoxidated from both faces, with the hemiacetal’s OH group assuming the syn configuration to the respective epoxides, presumably due to intramolecular hydrogen bonding.

Diepoxyabietane diterpenoid roscotane D was recently isolated from the whole plants of Kaempferia roscoeana.59 Again, there is very little information in the proton spectrum to suspect misassignment. However, accurate calculations of all three NMR parameter sets for the original structure and alternative candidates, i.e., proton and carbon chemical shifts, and proton SSCCs, reveal that the correct structure for...
roscotane D is its 13,14-epimer, Figure 19. The other candidates, i.e., epi-7,8 or epi-7,8,13,14, produced inferior matches for the experimental data.

An unusual endoperoxide containing the oxirane moiety, acremine P, was recently isolated from the fungus *Acremonium persicinum*. Its calculated spectra do not match the experiment, rmsd(δ_C) > 9 ppm. As shown in Figure 20, the largest two deviations from the calculated values were for C2 (Δδ_{exp}−calc = 102.4−121.8 = −19.4 ppm) and C7 (Δδ_{exp}−calc = 95.0−72.3 = 22.7 ppm). Given the experimental chemical shift of C2, we hypothesized that acremine P could be a 3-alkoxy-substituted enone, epoxyserinone A, shown for comparison—see text.

In such “congested” steroids, positioning of the oxirane oxygen on the opposite face of the polycyclic framework often causes a ripple effect on computed chemical shifts of the surrounding atoms for various reasons, including profound conformational changes. In this particular case, the largest deviation in the 13C chemical shifts computed for the original structures—ca. 10 ppm—was found for Me28, leaving no doubt that the structures are misassigned.

Finally, diterpenoid γ-lactone of briarane structural type, juncenolide G, was isolated from the gorgonian coral *Juncella juncea*, Figure 22 (the authors’ original structure drawing style is preserved for consistency, although the configuration at some carbon atoms, especially C8, is difficult to discern).
DU8+ computations revealed that the structure of juncenolide G is misassigned, rmsd($\Delta\delta$) = 3.7 ppm. Large deviations in computed $^{13}$C chemical shifts were observed for the C2–6 fragment, indicating potential misassignment of the 3,4-epoxy moiety. A number of candidate structures were analyzed to confirm this hypothesis. On the basis of this analysis, the structure of juncenolide G is revised to its epi-3,4 (still trans) stereoisomer.

**CONCLUSIONS**

As a fast and accurate method for NMR computations, DU8+ offers expeditious structure validation or revision for large collections of natural products. With this computational tool, we analyzed a number of NPs containing the oxirane moiety and revised 21 structures. Judging by this relatively high rate of misassignment, the presence of an oxirane moiety in natural products constitutes an additional challenge for structure elucidation. We cannot offer a statistically rigorous estimate of the rate of misassignment in naturally occurring epoxides, as our initial selection of natural epoxides from the literature is arbitrary. However, as we examined approximately 100 compounds, the rate of misassignment in the examined subset of natural epoxides exceeded 20%, which is slightly higher than our earlier observations of 13–15% for the rate of misassignment of other natural products.

However statistically imperfect, these misassignment rates are of concern. DFT computations of the ubiquitous 1D NMR spectra, presently available to the practitioners in the field, could help alleviate the majority of these misassignments.

As we noted before, another additional difficulty of structure validation/revision is a high rate of typos in the reported NMR data. This underscores the importance of dissemination of the original NMR data (i.e., FID data deposited in a digital format suitable for subsequent analysis). There have been several calls for action, the latest being coordinated by Guido Pauli under the community project on structural correctness.

**ASSOCIATED CONTENT**

- **Supporting Information**
  
  The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.8b01027.

  Computational details (PDF)

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**Notes**

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**REFERENCES**


(22) The following linear conversion of $^{13}$C isotropic magnetic shielding values, $\delta = 201.6–1.042$, was used for chloroform solutions; additional linear scaling of the calculated chemical shifts is applied to better match the experimental values in more polar solvents, especially DMSO. Such additional linear scaling is well precedent in the literature. For example, it worked well for Rychnovsky in the high-profile revision of hexacyclon. At the same time, we are in the process of developing the generalized linear scaling for specific solvents. For a description of “generalized linear scaling”, see ref 13.


(29) For calls to deposit original FIDs to alleviate errors in reporting experimental data, see: (a) Pauli, G. F.; Niemitz, M.; Bisson, J.; Lodewyk, M. W.; Soldi, C.; Shaw, J. T.; Tantillo, D. J.; Saya, J. M.;


(45) (48) Ho, S.；Zhou, L.; Zhang, G.-L. Chemical constituents of *Nouelia insignis* from the relative energy, even though it is not an energy minimum; see the Supporting Information.


(62) The only remaining mystery for acrmine P is that the authors catalytically hydrogenated it with H2 over Pd/C producing acrmine A, a known NP which has a C3–C7 bond. As the catalytic hydrogenation required two attempts on an initial amount of 0.7 mg of acrmine P with subsequent HPLC purification, one wonders if the identity of the hydrogenation product was misinterpreted.

