Structure Validation of Complex Natural Products: Time to Change the Paradigm. What did Synthesis of Alstofolinine A Prove?

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ABSTRACT: Total synthesis has been an effective and broadly practiced approach for structure validation (or revision) of complex natural products. It appears that computational methods for structure elucidation are gradually becoming a better alternative; faster and more reliable. The case of alstofolinine A.

Computer-aided methods for structure elucidation of complex natural products are becoming faster, more accurate, and more user-friendly. Synthesis of natural products will always be valuable for moving synthetic methodology forward, and also for offering an alternative supply of biologically active molecules often available only from scarce natural sources. However, when it comes to structure validation or revision, computational tools are quickly evolving as a better, cost-effective alternative.[1,2,3]

Recently reported synthesis of (–)-alstofolinine A employs several creative synthetic solutions, including the underutilized *aza*-Achmatowicz rearrangement followed by indole nucleophilic cyclization.[4] It achieves the target compound, matching perfectly the ^{1}H and ^{13}C NMR data obtained by the Kam lab in the original isolation of this natural product from the stem-bark and leaf extracts of the Malayan *Alstonia macrophylla* in 2014.[5] However, our *DU8+* computational analysis[6,7] of the presented NMR data reveals that these data do not support the structure of (–)-alstofolinine A synthesized in ref.[4] (i.e. the shown *endo*-butanolide), as it gives a poor match for the calculated ^{13}C and ^{1}H NMR chemical shifts and proton spin-spin coupling constants: *rmsd*(δ_C) = 2.50 ppm, *rmsd*(δ_H) = 0.20 ppm, and *rmsd*(J_{HH}) = 3.82 Hz, Figure 1 (note that Figure 1 deals with diastereomers, no absolute configuration is implied. For discussion of the absolute configuration see Figure 4).

Which diastereomer? All three rmsd values for the calculated data matched the shown *exo*-butanolide structure much better: *rmsd*(δ_C) = 1.38 ppm, *rmsd*(δ_H) = 0.12 ppm, *rmsd*(J_{HH}) = 0.38 Hz, leaving no doubt that this is the correct diastereomer.

This predicament necessitated a critical analysis of the synthetic path to the target alstofolinine A. The stereochemical outcome of the hydrogenation step **12** → **13** shown in Figure 1 was examined. The most plausible explanation is that the Pd/C hydrogenation of butenolide **12** occurs not from the *exo*- but rather from the *endo*-face, producing the *exo*-butanolide isomer of precursor **13**. This hypothesis is supported by calculated ^{13}C NMR chemical shifts for both precursor **13** and its *exo*-diastereomer. The match is better for the exo-isomer, *rmsd*(δ_C) = 1.27 ppm, than for the reported *endo*-isomer **13**, *rmsd*(δ_C) = 1.86 ppm.

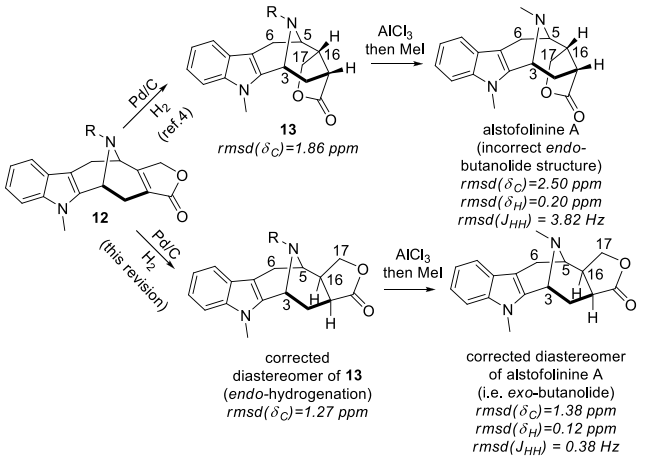

Figure 1. Last two steps in the total synthesis (ref.[4]). The corrected diastereomer of alstofolinine A implies that the hydrogenation of **12** occurs from the *endo*-face yielding the *exo*-butanolide product **13**.

Analysis of the proton spin-spin coupling constants (SSCCs) for alstofolinine A reveals additional irreconcilable differences for the *endo*-structure, while providing support for the correct *exo*-diastereomer, Figure 2. The most instructive discrepancy is revealed by the calculated values for $J_{H5\text{-}H16}$. In the experimental NMR data for alstofolinine A, proton H5 is described as a doublet with $J_{H5\text{-}H6a}$ = 6 Hz, indicating that the value of the second constant, $J_{H5\text{-}H16}$, is small. For the correct *exo*-structure the value of $J_{H5\text{-}H16}$ is calculated to be small indeed, 1.3 Hz. However, in the incorrect *endo* isomer this SSCC is calculated at 7.5 Hz, which is not observed experimentally. Also, for the correct *exo*-isomer, both $J_{H16\text{-}H17a}$ and $J_{H16\text{-}H17b}$ are matching nicely with the calculated values, while for the incorrect *endo*-isomer the value of $J_{H16\text{-}H17b}$ deviates by > 11 Hz.

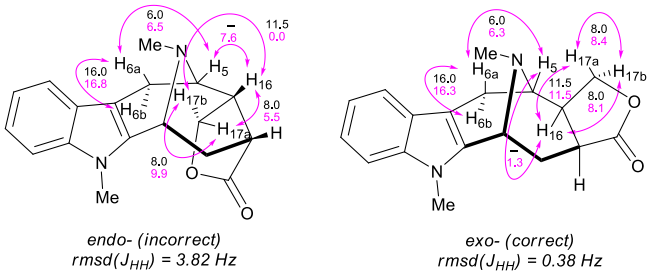

Figure 2. Most informative proton spin-spin coupling constants for the *exo*- and *endo*-candidate diastereomers; experimental values (black) are above calculated (magenta); rmsd values (Hz) for all nine reported SSCCs are also shown.

The combined *DU8+* calculated data establishes the structure of alstofolinine A as the *exo*-butanolide diastereomer, with the error most likely originating in the incorrect assumption about the facial selectivity of the Pd-catalyzed hydrogenation step.

A related question is whether the natural product was initially mischaracterized upon its isolation. We do not believe that this is the case, although somewhat esoteric depiction of the bridgehead protons in the original isolation paper may have contributed to the confusion, Figure 3A. Perhaps a more explicit and concise drawing convention is required for specifying the bridgehead stereochemical configuration unambiguously.[8] Admittedly, this way of depicting configuration of bicyclo[m.n.1] compounds (i.e. when the single atom bridge is located on the same face of the molecule as the "hedged" bridgehead hydrogen atoms) is adopted by a number of natural product research groups. If this indeed is the convention, the original isolation structure should be rewritten as shown in Figure 3B.

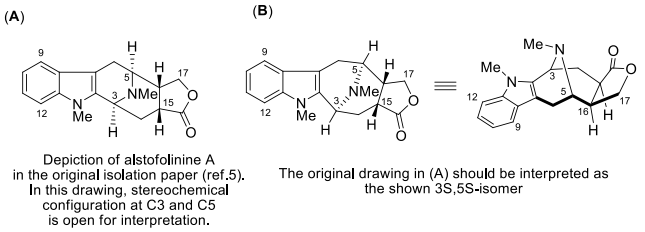

Figure 3. Depiction of the bridgehead stereochemical configuration used in the original isolation paper by Kam et al. (A); and its more conventional interpretations (B).

Besides the fact that the natural product is an *exo*-butanolide, as the calculations predict, it represents the *enantiomer* of the *exo*-corrected synthetic alstofolinine A.

Which enantiomer? In the original assignment, Kam and co-workers correctly relied on the similarity between the newly isolated (–)-alstofolinine A and macroline-type indole alkaloids. Both the natural and synthetic alstofolinine A have nearly identical optical rotation data: $[\alpha]_D$ = -104° (*c* = 0.36, $CHCl_3$) and $[\alpha]_D$ = -108° (*c* = 0.12, $CHCl_3$), respectively, suggesting that the synthetic sample has the same absolute configuration. To resolve this discrepancy, we calculated the $[\alpha]_D$ values for both enantiomers of the *exo*-diastereomer of alstofolinine A at the B3LYP/6-311++G(2d,2p)//B3LYP/6-311+(d,p) level of DFT theory. Figure 4 shows that these $[\alpha]_D$ calculations better match presumed original absolute configuration proposed by Kam et al., not its enantiomer, as the *exo*-corrected synthetic structure would suggest. Calculations for the middle, i.e. synthetic *endo*-structure in Figure 4 implies that the *exo*/*endo* butanolide moiety does not override the optical rotation of the macroline core.

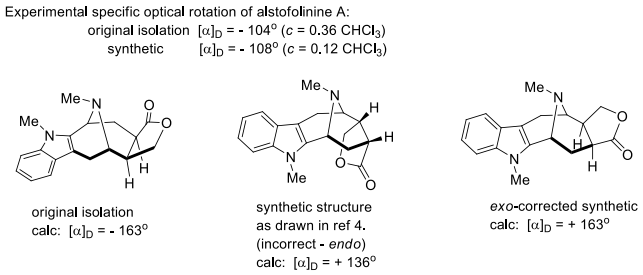

Figure 4. Experimental and calculated (B3LYP/6-311++G(2d,2p)//B3LYP/6-311+(d,p)) optical rotation for candidate structures.

Analysis of the asymmetric total synthesis revealed a potential source for error, see Figure 5. In the synthetic sequence the critical stereogenic center is introduced via the reaction of ketone **8** with Ellman's sulfinamide, followed by the reduction of the imine with (+)-diisopinocampheylborane to yield sulfonamide **9** with the *S*-configuration (original numbering of compounds in the synthetic paper is preserved). We do not have a reason to doubt the stereochemical outcome of this time-proven amination. However, we noticed that the *aza*-Achmatowicz product **6g** was depicted with the inversion of configuration at the C(N) stereocenter (i.e. *R*-configuration). This produced the wrong enantiomer of the bicyclic macroline core structure, which was carried through the rest of the synthetic sequence. This was compounded by the incorrect facial diastereoselectivity of the hydrogenation step.

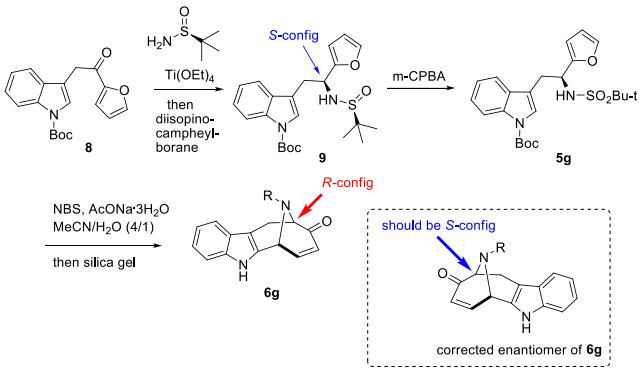

Figure 5. Introduction of the critical stereogenic center with Ellman's sulfonamide and a possible problem with the stereochemical outcome of the *aza*-Achmatowicz reaction (original numbering of compounds).

To conclude, based on our computational analysis, we confirm the relative and absolute configuration of (–)-alstofolinine A assigned by Kam et al. and as shown in Figure 6.

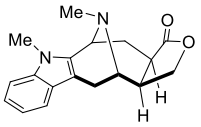

Figure 6. Confirmed correct relative and absolute configuration of (–)-alstofolinine A as reported by Kam et al.

The lesson learned here is that independent total synthesis does not guarantee 100% error-free structure validation, and that practitioners in the field should embrace modern computational tools for predicting NMR spectra (and other physical observables). These increasingly user-friendly computational tools are now fast and sufficiently accurate in most cases to expeditiously detect a misassignment and avoid errors.

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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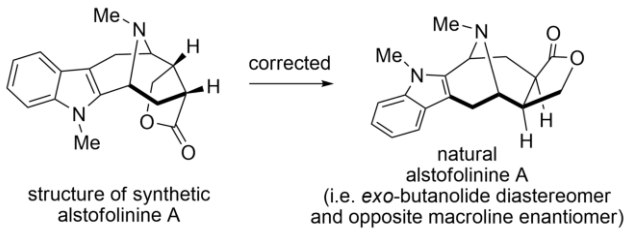

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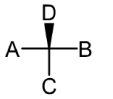
(7) *DU8+* uses empirical corrections to circumvent computing 'difficult' components and substantially accelerate the computations. For example, empirical corrections are used to avoid expensive calculations of relativistic contributions to magnetic shielding arising from the heavy atom effect, etc. Rovibrational corrections on chemical shifts are also accounted for via empirical corrections under the *DU8+* formalism. These effects could be computed explicitly, but require much more expensive molecular dynamics computations, see for example Kwan, E. E.; Liu, R. Y. Enhancing NMR Prediction for Organic Compounds Using Molecular Dynamics. *J. Chem. Theory Comput.* **2015**, *11*, 5083.

(8) One reviewer helpfully pointed to an IUPAC document http://publications.iupac.org/pac/2006/pdf/7810x1897.pdf including a relevant case of "Three plain bonds and one wedged bond, with one pair of plain bonds separated by 180° or more and the wedged bond positioned within that largest space between plain bonds" (ST1.1.4):



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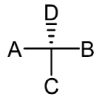

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