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# Structure Revision of an Acorane Sesquiterpene Cordycepol A

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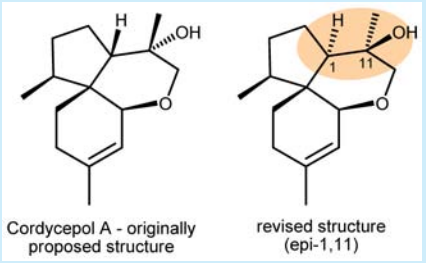
Supporting Information

**ABSTRACT:** Structure revision of a recently reported spirodecane sesquiterpene, cordycepol A, from fungi *Cordyceps ophioglossoides* was enabled by fast and accurate calculations of nuclear spin−spin coupling constants (SSCCs) with a *relativistic force field* (*DU8c*) parametric method. Two other reported cordycepols, B and C, are also identified as misassigned. Calculations of accurate SSCCs, which contain a wealth of structural information, offer a chemically intuitive tool for structure elucidation, rendering the whole structure revision process more guided and intentional, while augmenting in a synergistic way the calculations of chemical shifts.




Four sesquiterpenes, cordycepols A−C, and cordycol were relatively recently isolated from fungi *Cordyceps ophioglossoides*.[1] They were deemed promising lead compounds for treating human hepatic carcinoma. Cordycepols A−C were assigned a "new unusual" spiro[4.5]decane core (although we note that sesquiterpenes with an acorane skeleton[2] were known for a long time). The proposed structure of cordycepol A is shown in Figure 1. Analysis of calculated $^{13}C$ chemical shifts[3]

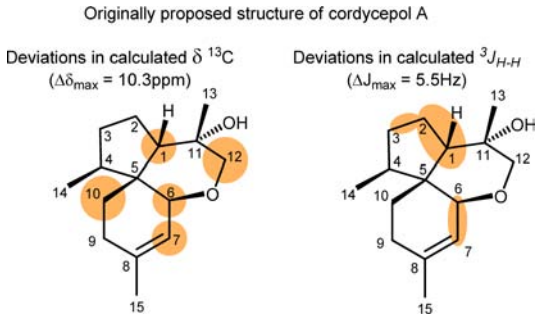



**Figure 1.** Originally proposed structure of cordycepol A: computed deviations in $^{13}C$ chemical shifts, left, and vicinal spin−spin coupling constants, right (the size of the orange blob corresponds to the magnitude of deviation). See also Table 1.

and, most importantly, proton spin−spin coupling constants (SSCCs) revealed a number of discrepancies graphically mapped on the originally proposed structure in Figure 1, with the calculated chemical shifts of C(10) and C(12) deviating most, by −10.3 and 9.7 ppm respectively, and both $^3J_{1-2a}$ and $^3J_{1-2b}$ deviating by 4.5 and 5.5 Hz. As our *relativistic force field* (*rff*) method[4] for computing nuclear SSCCs offers high accuracy with maximum deviations normally falling below 1 Hz, we concluded that the originally assigned structure is incorrect.

DFT calculations of $^{13}C$ chemical shifts[5] proved critical in several high profile structure revisions, for example, hexacyclinol by Rychnovsky,[6a] or aquatolide and nobilisitine A by Tantillo.[6b,c] Computations of *nuclear spin−spin coupling constants* are also available, but expensive.[7] Bally and Rablen have pioneered the first practical approach to fast evaluation of SSCCs via scaling of *easy* Fermi contacts,[8] as there was a growing consensus that the Fermi contact mechanism dominates nuclear spin scalar coupling.[9] Capitalizing on these developments we recently introduced a multiparametric *rff* method[4] which offers fast and accurate scaling of Fermi contacts with the help of NBO hybridization parameters.[10]

Utilization of the *rff*-computed SSCCs, which contain a wealth of structural information, in conjunction with the $^{13}C$ chemical shifts serves as a reliable, dual-criteria structure elucidation tool. We suggest that this dual-criteria approach allows for safe and justifiable utilization of less expensive theory levels and lighter basis sets, effectively accelerating the process of structural/stereochemical assignment. The proton chemical shifts could serve as an auxiliary third criterion, although $^1H$ shifts are excessively conditions-/solvent-dependent.[11] In this letter we apply this aggregate approach to the reassignment of stereochemistry of cordycepol A.

Proton H1 in cordycepol A has two experimental SSCCs, 13.4 and 7.1 Hz. The *rff*-calculated values for these constants are 8.9 and 1.6 Hz for the proposed structure. The putative C1-epimer **1**, Figure 2, gave somewhat better agreement with

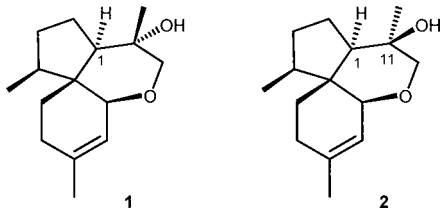



**Figure 2.** Alternative candidate structures for cordycepol A: *epi*-1 (**1**) and *epi*-1,11 (**2**).

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experimental SSCCs but the match of $^{13}C$ chemical shifts, while improved from rmsd of 5.8 ppm to 2.54 ppm, still was not perfect, with C12 deviating by more than 5 ppm. This indicated that the original stereochemical assignment of the tertiary alcohol at C11 was also incorrect. This stereogenic center has no protons and therefore exerts only an indirect effect on proton spin−spin coupling.

For the *epi*-1,11 structural candidate **2** shown in Figure 2 we identified four lowest energy conformers, 0.00, 1.72, 1.80, and 3.40 kcal/mol; populations: 90.4%, 5.0%, 4.3%, 0.3% according to their ZPE-corrected DFT energies, Figure 3.

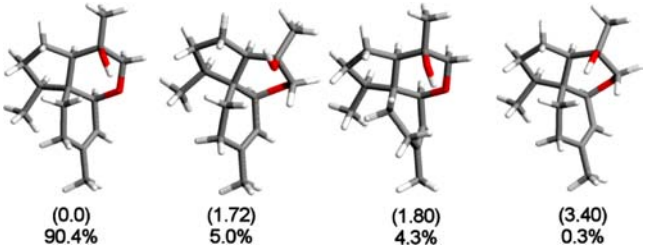


**Figure 3.** Four lowest energy conformers for the candidate structure **2** (relative kcal/mol in parentheses); b3lyp/6-31G(d), ZPE-corr.

Energy-weighted mixing of these four conformers produced an excellent match with the experimental NMR data. The $^{13}C$ chemical shifts were matched with an rmsd of 1.54 ppm. The $^{1}H$ chemical shifts gave 0.13 ppm accuracy, and the SSCCs were predicted with rmsd = 0.63 Hz. The largest deviation, $\Delta J$ = 1.36 Hz, was due to $^{3}J_{6-7}$ which was also poorly correlated in the structure **1**. We do not have a good explanation for this >1 Hz deviation, although we do not believe that this is indicative of the C6-epimer, because the same $^{3}J_{6-7}$ in the *epi*-6 stereoisomer deviated by 1.26 Hz from the experimental value. Omission of $^{3}J_{6-7}$ improves the rmsd to 0.39 Hz.[12,13]

Table 1 summarizes the results of our calculations for both the originally proposed and the revised structures of cordycepol A. The weightings of the two lowest energy conformers for the originally proposed structure were 99.9% and 0.1% based on zpe-corrected gas phase b3lyp/6-31G(d) energies (the chemical shifts are calculated at the mPW1PW91/6-311+G-(d,p) level of DFT theory, and spin−spin coupling constants are obtained with *rff* DU8c; for details see Supporting Information). The data presented in Table 1 leave little doubt that the 1,11-epimer **2** shown in Figure 2 is indeed the correct structure of cordycepol A.[14]

This structure correction of cordycepol A underscores our philosophy of computational reassignment of structures/stereochemistry. Providing high structural information content, fast and accurate calculations of SSCCs allow for expeditious screening of a large number of potential structure candidates and their conformations in a very practical amount of time. Comparison of all three experimental data sets ($J$'s, $\delta_H$, and $\delta_C$) reduces the reliance on any one of these data sets (chemical shifts or SSCCs) and, therefore, allows for the utilization of lighter basis sets and, generally, faster computations. In our experience, the geometry optimization (B3LYP/6-31G(d)) calculations of $^{1}H$ and $^{13}C$ chemical shifts (mPW1PW91/6-311+G(d,p)) and calculations of Fermi contacts (DU8c[4]) for a molecule of cordycepol A size take under 45 min on a 16 core node of a Linux cluster (including not only $J_{HH}$ but also a full set of $J_{CH}$ constants helpful for analyzing discrepancies in the HMBC data). This implies that several conformers of a candidate structure could be fully analyzed in parallel under an hour, without the need for computing the chemical shifts or SSCCs at a much higher level of theory, which could take days if not weeks for large organic molecules.

**Table 1. Experimental and Calculated NMR $J$'s and $\delta$'s for the Original and Revised Structures of Cordycepol A[a]**

| | exp | Original structure calc | Δ | Revised structure (**2**) calc | Δ |
|---|---|---|---|---|---|
| *J(H-H), Hz* | | | | | |
| $J_{1-2b}$ | 7.1 | 1.60 | 5.50 | 6.90 | 0.20 |
| $J_{1-2a}$ | 13.4 | 8.93 | 4.47 | 13.54 | -0.14 |
| $J_{3a-3b}$ | 13.0 | 12.51 | 0.49 | 12.96 | 0.04 |
| $J_{2a-3a}$ | 6.8 | 4.40 | 2.40 | 6.61 | 0.19 |
| $J_{3a-4}$ | 9.2 | 8.93 | 0.27 | 8.86 | 0.34 |
| $J_{6-7}$ | 4.5 | 2.73 | 1.77 | 5.86 | -1.36 |
| $J_{12a-12b}$ | 12.0 | 11.22 | 0.78 | 11.16 | 0.84 |
| | | *rmsd = 2.93 Hz* | | *rmsd = 0.63Hz* | |
| *$^{1}H$ δ, ppm* | | | | | |
| H(1) | 1.35 | 1.83 | -0.48 | 1.27 | 0.08 |
| H(2a) | 1.67 | 1.75 | -0.08 | 1.79 | -0.12 |
| H(2b) | 1.58 | 2.02 | -0.44 | 1.66 | -0.08 |
| H(3a) | 1.90 | 2.03 | -0.13 | 1.94 | -0.04 |
| H(3b) | 1.39 | 1.27 | 0.12 | 1.50 | -0.11 |
| H(4) | 1.54 | 2.33 | -0.79 | 1.48 | 0.06 |
| H(6) | 3.33 | 3.99 | -0.66 | 3.30 | 0.03 |
| H(7) | 5.69 | 5.12 | 0.57 | 5.82 | -0.13 |
| H(9a) | 2.13 | 1.81 | 0.32 | 2.25 | -0.12 |
| H(9b) | 2.04 | 2.16 | -0.12 | 2.08 | -0.04 |
| H(10a) | 2.45 | 1.35 | 1.10 | 2.21 | 0.24 |
| H(10b) | 1.45 | 1.39 | 0.06 | 1.56 | -0.11 |
| H(12a) | 3.71 | 3.5 | 0.21 | 3.46 | 0.25 |
| H(12b) | 3.26 | 3.02 | 0.24 | 3.13 | 0.13 |
| H(13) | 1.09 | 0.94 | 0.15 | 1.03 | 0.06 |
| H(14) | 0.99 | 0.93 | 0.06 | 1.03 | -0.04 |
| H(15) | 1.69 | 1.88 | -0.19 | 1.90 | -0.21 |
| | | *rmsd = 0.44 ppm* | | *rmsd = 0.13 ppm* | |
| *$^{13}C$ δ, ppm* | | | | | |
| C(1) | 56.2 | 47.04 | 9.16 | 56.26 | -0.06 |
| C(2) | 19.2 | 22.85 | -3.65 | 20.11 | -0.91 |
| C(3) | 30.0 | 30.83 | -0.83 | 30.8 | -0.8 |
| C(4) | 43.7 | 40.39 | 3.31 | 46.09 | -2.39 |
| C(5) | 42.5 | 45.78 | -3.28 | 44.31 | -1.81 |
| C(6) | 81.3 | 72.71 | 8.59 | 80.55 | 0.75 |
| C(7) | 120.1 | 128.87 | -8.77 | 123.97 | -3.87 |
| C(8) | 142.1 | 144.93 | -2.83 | 144.77 | -2.67 |
| C(9) | 29.4 | 27.64 | 1.76 | 29.11 | 0.29 |
| C(10) | 17.6 | 26.09 | -8.49 | 19.26 | -1.66 |
| C(11) | 71.3 | 67.76 | 3.54 | 70.58 | 0.72 |
| C(12) | 77.9 | 67.46 | 10.44 | 76.63 | 1.27 |
| C(13) | 25.1 | 21.22 | 3.88 | 21.19 | 2.11 |
| C(14) | 16.3 | 12.49 | 3.81 | 14.61 | 1.69 |
| C(15) | 23.3 | 22.93 | 0.37 | 23.14 | 1.96 |
| | | *rmsd = 5.80 ppm* | | *rmsd = 1.54 ppm* | |

[a]Red color highlights large discrepancies in calculated values: $|\Delta J| > 1$ Hz, $|\Delta\delta_H| > 0.2$ ppm, $|\Delta\delta_C| > 3$ ppm.

The proposed structures of the other two reported cordycepols, B and C, have similar spiro[4.5]decane cores found in acorane-type sesquiterpenes, but lack a pyran ring, Figure 4.

Multiple peak overlaps in the experimental $^{1}H$ NMR spectra of cordycepols B and C prevented the authors from reporting SSCCs, critical for assigning structure and stereochemistry. Based on our analysis of $^{13}C$ chemical shifts, we are confident that both structures are also misassigned. However, without the experimental SSCCs it would be challenging to propose a plausible structure revision. The only important SSCC reported



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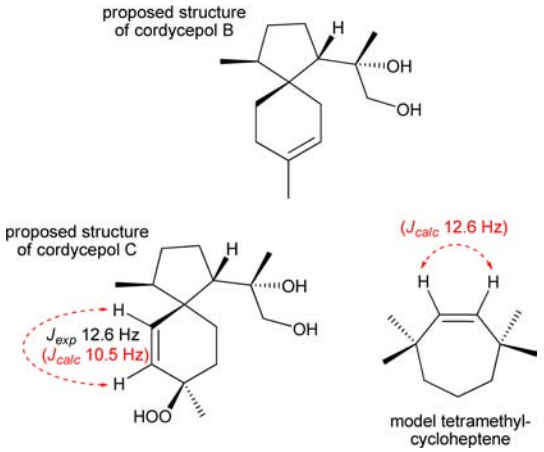


**Figure 4.** Proposed structures of cordycepols B and C. The reported HC═CH SSCC for cordycepol C, $^3J_{exp}$ = 12.6 Hz, better matches the value calculated for a model tetramethylcycloheptene.

for the core of cordycepol C was the vicinal constant for the cyclohexenyl HC═CH moiety, $^3J_{exp}$ = 12.6 Hz. Its calculated value of 10.5 Hz is consistent with the expectations for cyclohexenes, but does not match the experimental data for the proposed structure. Based on this sole SSCC it is highly unlikely that the natural cordycepol C has a cyclohexene moiety. The experimental value matches perfectly the calculated HC═CH constant for a model 3,3,7,7-tetramethylcycloheptene ($^3J_{calc}$ = 12.6 Hz), Figure 4. It is plausible that cordycepol C belongs to hydroazulene-type sesquiterpenes, i.e. derivatives of bicyclo[5.3.0]decane.

In conclusion, we have demonstrated that the structures of all three cordycepols A−C are misassigned. Based on combined chemical shift and SSCCs data we revised the structure of cordycepol A to structure **2**. We hypothesize that cordycepol C belongs to a different structural type−hydroazulene-type sesquiterpene.[15] On a separate note, this work underscores the importance of dissemination of the original NMR data (i.e., FID data 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.[16] This is particularly important for compounds such as cordycepols, which are deemed promising as therapeutic agents.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02341.

Computational details (PDF)

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### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

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(12) To test that the deviation in $J_{6-7}$ is not an artifact of *rff* scaling, the computations were rerun at a higher level of DFT theory, mPW1PW91/6-311+G(d,p)//b3lyp/6-311+G(d,p), using Gaussian's *spinspin* keyword, i.e. calculating the Fermi contacts and all other contributions to *J*, such as spin-dipolar as well as paramagnetic and diamagnetic spin−orbit coupling. This resulted in a very good match between the *J*-values calculated with two different methods. For example, for the major 90.4% conformer this calculation gave $J_{6-7}$ = 5.87 Hz, whereas the *rff* method gave the value of 5.90 Hz. Note that while the result was the same, *rff* calculations were three times faster.

(13) For extra certainty, we tested several additional stereoisomers, *epi*-4-, *epi*-1,4-, *epi*-1,4,11-, *epi*-1,6-, *epi*-1,6,11-, and also isomeric structures possessing a THF ring in place of the THP moiety. Their calculated NMR data did not match the experimental spectra.

(14) There are two discrepancies between the revised structure and the NOESY cross-peaks reported in ref 1. We attributed this to a considerable crowding of multiple protons in the 1−2 ppm area of the spectrum. (a) The reported H1−Me14 cross-peak: proton H1 (1.35 ppm) has an overlap with the high field proton belonging to methylene $CH_2$(3), 1.37−1.41 ppm. The observed NOESY cross-peak could be due to H3−Me14, which are *syn*- to each other in both revised and the original structures. (b) The reported H1−Me15 cross-peak: according to the original calculated structure, the distance between H1 and Me15 exceeds 4.3 Å, while the distance H1−H9 is less than 2.2 Å; yet the cross-peak H1−H9 is not observed (the lack of the H1−H9 cross-peak is actually consistent with the revised structure). Again, it is likely that the heavy overlap caused the misreporting of the H1−Me15 cross-peak. For example, Me15 at 1.69



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ppm is overlapping with $CH_2$(2) at 1.65−1.69 ppm; it is conceivable that the authors actually see the H1−H2 cross-peak which should be observed in both revised and the original structures.

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