Homodecoupled 1,1- and 1,n-ADEQUATE NMR experiments: Application to the structural elucidation of proton-deficient natural products

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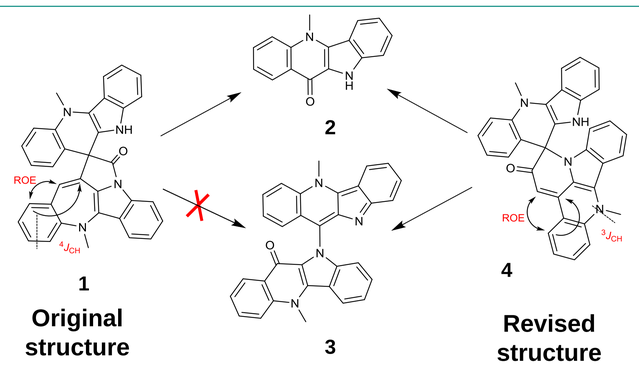
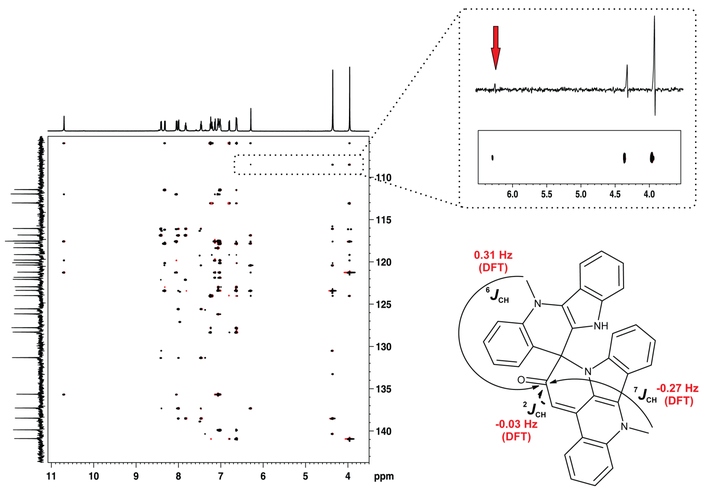
Abstract

Cryptospirolepine is a complex, indoloquinoline alkaloid that was isolated in late 1991 from extracts of the Ghanaian chewing stick, *Cryptolepis sanguinolenta*. When the structure was reported in 1993[1], the structure elucidation employed what were then state-of-the-art NMR methods and 3-mm micro-NMR probe technology. Using the NMR methods then available, there were no correlations observed to the carbonyl in the HMBC data that could be acquired. The structure elucidation predated the application of ^{1}H-^{15}N HMBC and ADEQUATE methods to natural products by several years. Computer-assisted structure elucidation, or CASE, methods were in their infancy and were incapable of dealing with a molecule of the complexity of cryptospirolepine. Hence, the assembly of the structure hinged on an ROE observed between a vinyl proton and one of the terminal resonances of a four-spin aromatic system, a putative and unusually strong $^{4}J_{CH}$ correlation to a quaternary carbon from the same aromatic proton, and the absence of any long-range heteronuclear correlation to the single carbonyl carbon. Nearly a decade later, several degradants were isolated from the original sealed NMR sample[2], one of which had a structure whose formation could not be rationalized from the reported structure of cryptospirolepine. In light of this history, we have interrogated a 700-µg voucher sample of cryptospirolepine using 1.7-mm MicroCryoProbe™ technology, 2 Hz optimized ^{1}H-^{13}C and ^{1}H-^{15}N LR-HSQMBC[3], newly developed 1,1- and 1,n-HD-ADEQUATE methods[4], DFT calculations, and modern CASE methods.

To accommodate the absence of any long-range correlation to the carbonyl and the presence of an ROE correlation in the original report[1], it was incorrectly assumed that the carbonyl would have to be at least four bonds removed from the nearest proton. The problem was an unusually strong $^{4}J_{CH}$ correlation and the inability to explain the formation of one of the degradants.



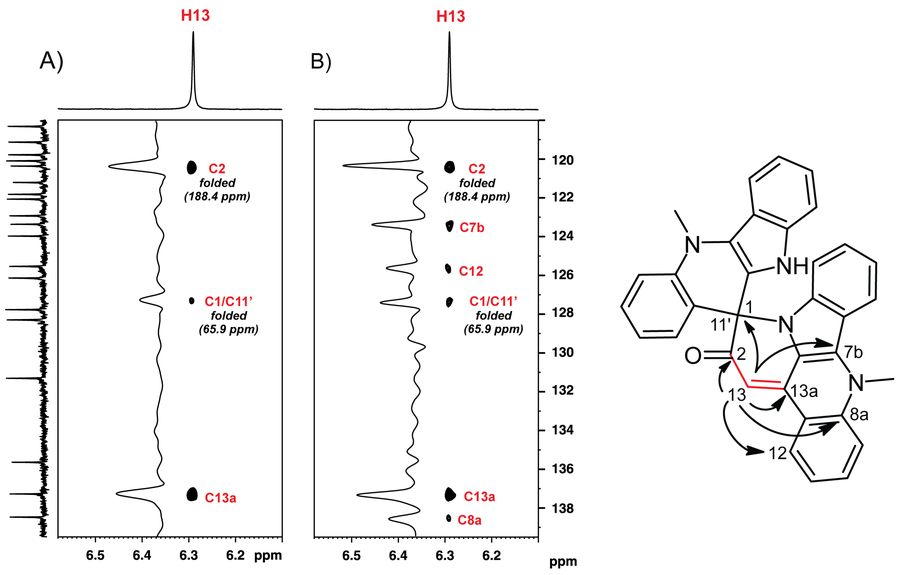
LR-HSQMBC



As noted above, a critical point from the original cryptospirolepine structure elucidation was the lack of any long-range heteronuclear correlation to the carbonyl. To further investigate whether any correlation to the carbonyl might be observable, the recently developed LR-HSQMBC experiment was acquired, optimized for 2-Hz correlations.[3]

From the 1D slice extracted at the folded carbonyl frequency (top right), two N-Me correlations are observed, along with a possible, very weak correlation from the vinyl proton to the carbonyl. However, the signal-to-noise ratio of the latter was quite low (~2:1) and therefore questionable.

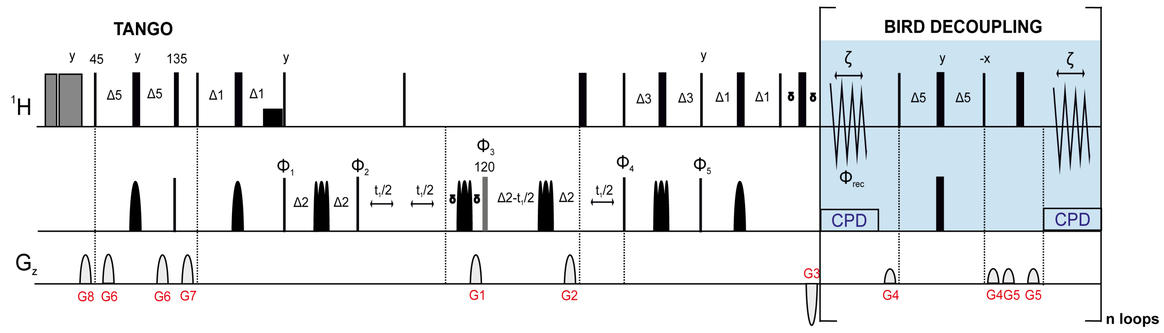
1,1- and 1,n-HD-ADEQUATE

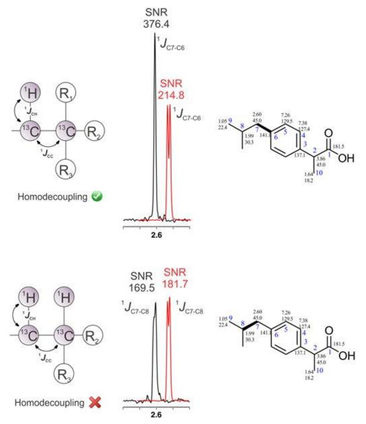


A) Segment of the 60-Hz optimized 1,1-HD-ADEQUATE spectrum that provided the critical data necessary to successfully revise the structure of cryptospirolepine. The vinyl carbon is flanked by the carbonyl and the quaternary carbons resonating at 188.4 and 137.3 ppm, respectively. Hence, the [7.5.5] central core of the pentacyclic portion of the molecule (**1**) becomes a [6.6.5] moiety (**4**). **B)** A 7-Hz optimized 1,n-HD-ADEQUATE experiment confirmed additional homonuclear ^{13}C-^{13}C correlations consistent with the revised structure.

Pulse Scheme: Homodecoupled 1,1 and 1,n-ADEQUATE

When "silent" resonances still remain after higher-sensitivity techniques fail, NMR experiments such as 1,1-ADEQUATE and 1,n-ADEQUATE can become a useful alternative. To circumvent the dearth of proton correlations to the carbonyl, a new homodecoupled 1,1-ADEQUATE (1,1-HD-ADEQUATE) experiment optimized for 60 Hz was acquired. As in the pure shift HSQC experiment, the modified 1,1-HD-ADEQUATE employs a series of BIRD modules during a "chunked" data acquisition.





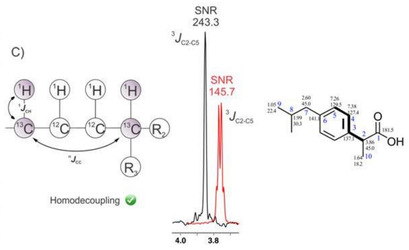
1,1-HD-ADEQUATE:

$^{13}C_A$**H**-$^{13}C_B$**H** will not decouple because the BIRD pulse cannot differentiate protons attached to ^{13}C carbons.

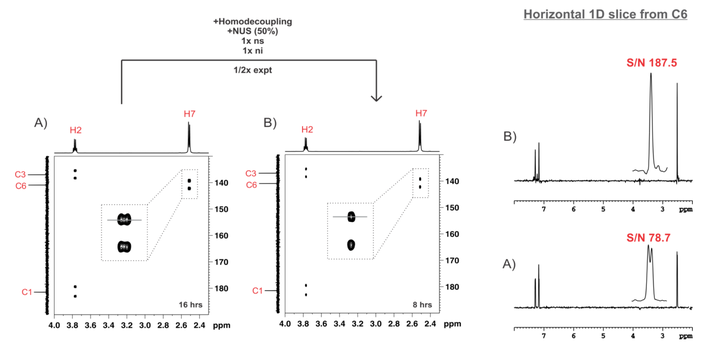
$^{13}C_A$**H**-$^{13}C_B$ will decouple since all other protons coupled to $^{13}C_A$**H** are attached to ^{12}C.

1,n-HD-ADEQUATE:

Longer-range correlations ($^{13}C_A$**H**-$^{12}C_B$-$^{12}C_C$-$^{13}C_D$**H**) will be decoupled except for the small long-range homonuclear proton-proton coupling between the remote protons on ^{13}C.



Measurement of J(CC) from J-modulated HD-ADEQUATE



Comparison of conventional *J*-modulated 1,1-ADEQUATE (panel A) and J-modulated 1,1-HD-ADEQUATE (panel B) spectra of ibuprofen. The spectrum shown in panel A was acquired with ni=256, with 96 transients/t_1 increment and a scaling factor K=10, giving an acquisition time of 16 h. In contrast, the data shown in panel be were acquired with 50% non-uniform sampling with 128/256 increments, 96 transients/t_1 increment and a scaling factor K = 10, giving an acquisition time of 8 h. As expected, the H7 doublet is collapsed for the C7-C6 correlation to a singlet in the spectrum shown in panel B (see discussion in Figure 2). Slices extracted at the F_1 chemical shift indicated by the horizontal line in the insets are compared in the right panel. Whereas the S/N ratio of the conventional spectrum gave a S/N ratio of 78.7:1, the non-uniformly sampled data gave a S/N ratio for the same resonance of 187.5:1 using only half the acquisition time.

Conclusions

- Homodecoupled 1,1-ADEQUATE (1,1-HD-ADEQUATE) provided crucial structural information that allowed the revision of the structure of cryptospirolepine. The revised structure is now consistent with the structures of the degradation products described in 2002[2]
- The extension of BID-based homonuclear decoupling to quaternary carbons in both the 1,1- and 1,n-ADEQUATE experiments[4] affords higher-sensitivity versions of these experiments that can be applied to challenging structure elucidation problems when more sensitive techniques fail
- The addition of 1,1-HD-ADEQUATE and 1,n-HD-ADEQUATE as input data into CASE program had a huge impact in terms of generation time and the number of structures proposed

References:

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2. Martin GE, Hadden CE, Russell DJ, et al. *J Heterocycl Chem.* 2002;39:1241-1250.
3. a) Williamson RT, Buevich AE, Martin GE, Parella T. *J Org Chem.* 2014;79:3887-3894. b) Williamson RT, Buevich AV, Martin GE. *Tet Lett.* 2014;55:3355-3356.
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Acknowledgments:

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