NMR Techniques

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Homodecoupled 1,1- and 1,n-ADEQUATE: Pivotal NMR Experiments for the Structure Revision of Cryptospirolepine**

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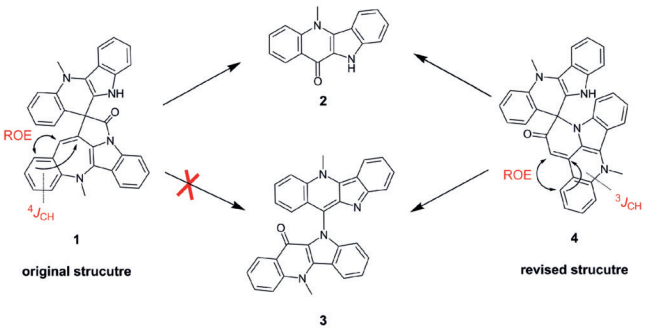
In memory of Albert N. Tackie

Abstract: *Cryptospirolepine is the most structurally complex alkaloid discovered and characterized thus far from any Cryptolepis specie. Characterization of several degradants of the original, sealed NMR sample a decade after the initial report called the validity of the originally proposed structure in question. We now report the development of improved, homodecoupled variants of the 1,1- and 1,n-ADEQUATE (HD-ADEQUATE) NMR experiments; utilization of these techniques was critical to successfully resolving long-standing structural questions associated with crytospirolepine.*

Recently, our group revisited persistent structural questions surrounding the Ghanian plant alkaloid cryptospirolepine (**1**), a severely sample-limited and proton-deficient indoloquinoline alkaloid isolated from *Cryptolepis sanguinolenta*.[1] The structure of this natural product was first reported in 1993 and the original structure characterization has been cited in excess of 60 times over the intervening decades. The original structure proposal was reported prior to the first application of ^{1}H-^{15}N HMBC[2,3] experiments for natural product structure elucidation and the structure determination effort was severely hampered by the absence of any long-range heteronuclear correlation to the carbonyl carbon irrespective of the optimization of the HMBC experiment. In the original work, dipolar couplings observed in a ROESY experiment required the vinyl proton to be in close proximity to one of the four-spin aromatic systems. In addition, the assigned structure attributed an intense response in the HMBC spectrum to a $^{4}J_{CH}$ correlation to one of the quaternary carbons. Nearly a decade later, several major degradants of cryptospirolepine, **2** and **3**, were isolated from the original sealed NMR sample in [D_6]DMSO (Scheme 1). In particular, one of these impurities, **3**, could not be mechanistically rationalized from the structure assigned in 1993.[4] With advances in cryoprobe technology, including 1.7 mm micro cryoprobes,[5] the development of long-range heteronuclear shift correlation methods such as LR-HSQMBC,[6] and improved homodecoupled variants of 1,1- and 1,n-ADEQUATE (HD-ADEQUATE) experiments described here, the timing was propitious to reinvestigate the structure of this complex alkaloid, **1**, which ultimately led to the revision of the structure, **4**.



Scheme 1. The original (**1**)[1] and revised (**4**) structures of cryptospirolepine and the structures of two degradants (**2** and **3**)[4] characterized in 2002.

ADEQUATE experiments provide visualization of carbon–carbon connectivity through an out-and-back-type transfer that relies on an initial and final $^{1}J_{CH}$ magnetization transfer with intervening evolution of homonuclear J_{CC} coupling pathways.[7–9] Hence, correlations for quaternary carbons in 1,1- ($^{1}J_{CC}$) and remote carbons in 1,n-ADEQUATE

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($^nJ_{CC}$, where $n = 2$–4) are amenable to multiplet simplification of the detected proton thanks to BIRD-based homonuclear decoupling techniques as previously reported for the HSQC experiment.[10–16] Since the ADEQUATE experiments are relatively insensitive compared to HSQC, sensitivity increases in the range typically realized by applying broadband homonuclear decoupling (40–60%), could reduce data acquisition times by up to half or more, enhancing the utility and viability of these techniques in a wide variety of challenging and heretofore inaccessible structure elucidation problems.

The gains in sensitivity realized for correlations between protonated and adjacent quaternary carbon resonances in 1,1-ADEQUATE spectra[20] and for long-range correlations in the 1,n-ADEQUATE experiment through homodecoupling are especially important in scarce sample situations such as the present example.

The pulse sequence schematic for the HD-ADEQUATE experiments is shown in Figure 1. As in the standard 1,1- and 1,n-ADEQUATE experiments, the corresponding partially homodecoupled variants differ only in the duration of the delays used for $^1J_{CC}$ or $^nJ_{CC}$ transfers, which are typically optimized in the ranges of 30–60 Hz and 3–7 Hz, respectively.[7–9] Results of the decoupling afforded by the HD-ADEQUATE experiments are summarized for ibuprofen in Figure 2. Briefly, correlations between protonated and adjacent quaternary carbons (^{13}CH–^{13}C) will be decoupled (Figure 2A). Correlations between adjacent protonated carbons (^{13}CH–^{13}CH) will not be decoupled from each other analogous to the anisochronous protons of a geminal methylene in a pure shift HSQC spectrum (Figure 2B). Long-range correlations ($^2J_{CC}$, ^{13}CH–^{12}C–^{13}CH and $^3J_{CC}$, ^{13}CH–^{12}C–^{12}C–^{13}C) will be decoupled except for the small homonuclear proton–proton coupling between the remote proton(s) on ^{13}C, which generally will not be observed given the typical resolution in this type of experiment (see Figure 2C). The results obtained with the 1,1-HD-ADEQUATE experiment for a pair of correlations from the spectrum of **4** are shown in Figure 3A–C and 3E. Results obtained with the 1,n-HD-ADEQUATE experiment performed on a sample of **4** are shown in Figure 3D and 3F. Analogous to the pure shift HSQC experiment, the modified HD-ADEQUATE employs a series of BIRD modules[21] during a "chunked" data acquisition.[13] Due to the nature of this data acquisition strategy, and the increased number of RF pulses, an increase in the level of artifacts is expected. This "noise" will primarily be associated with ^{12}C-bound protons and protons bound to an isolated ^{13}C atom while the signals of interest emanate from molecules with ^{13}C coupled atom pairs (^{1}H^{13}C–^{13}C or ^{1}H^{13}C–(^{12}C)$_n$–^{13}C where $n = 2$–3).[7–9] Hence, signals from a ^{1}H–^{12}C or isolated ^{1}H–^{13}C moieties should be suppressed as much as possible to minimize artifacts. In order to achieve this goal, additional "building blocks" have been inserted in the standard ADEQUATE pulse sequence. These include a purge element (2.5 ms(x) 5 ms(y) at 10 kHz RF field followed by a spoil gradient) prior to the relaxation delay to destroy all undesired components of magnetization. We also incorporated a TANGO[22] gradient element to excite and dephase ^{12}C bound protons, while leaving ^{13}C bound protons unaffected. Finally, a 1 ms high power trim pulse was applied at the end of the initial INEPT transfer step to help destroy any remaining undesired transverse magnetization. Full details of the pulse sequence are presented in the Supporting Information.

In the case of cryptospirolepine (**4**), neither 4 Hz nor 2 Hz optimized LR-HSQMBC[6] data afforded structurally relevant correlations to the lone carbonyl from the vinyl proton. There was a weak hint of a correlation (s/n ≈ 2:1) in the 2 Hz optimized spectrum and correlations were observed from both *N*-methyl groups to this key carbon. Unfortunately, the "length" of these $^nJ_{CH}$ correlations cannot be deduced from the LR-HSQMBC data. There is no way to determine, that the weak potential correlation from H13 to C2 was via $^2J_{CH}$. Other structural segments assembled in the 1993 report[1] were reconfirmed with modern data (see Supporting Information). The dearth of correlations to the carbonyl, a mass-limited sample (≈2 mg), and limited solubility (≈700 μg/35 μL [D$_6$]DMSO) prompted us to employ a newly developed 1,1-HD-ADEQUATE NMR experiment to provide unambiguous connectivities for these partial structures.

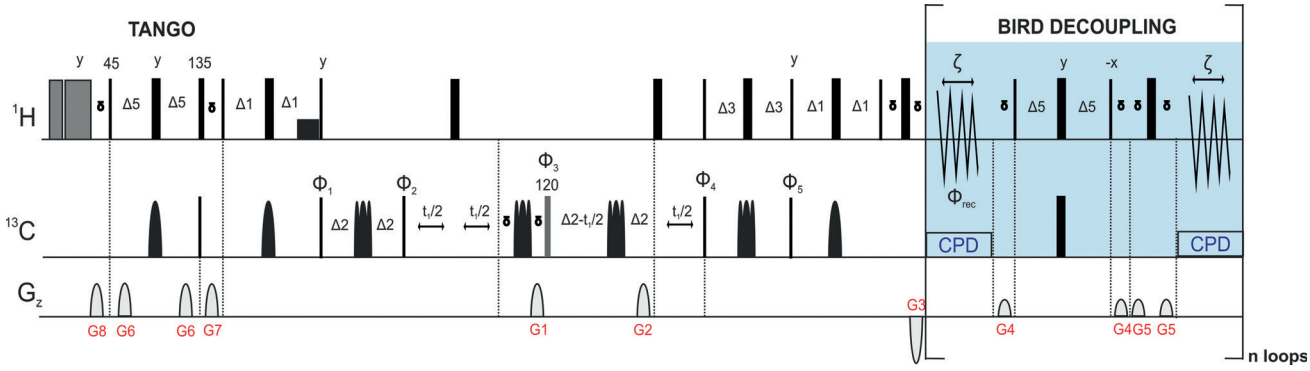

Figure 1. Pulse sequence for 1,1- and 1,n-HD-ADEQUATE modified to suppress noise and artifacts in the spectrum in addition to providing partial proton homonuclear decoupling. Narrow black bars represent 90° pulses unless otherwise labeled; wide black bars represent 180° pulses. The blue shaded region of the pulse sequence denotes the BIRD-based homonuclear decoupling scheme. Sine bell shaped bars correspond to shaped ^{13}C pulses. Full details for the pulse sequence including phase cycling, pulse shapes, gradient strengths, and other key parameters are given in the Supporting Information.

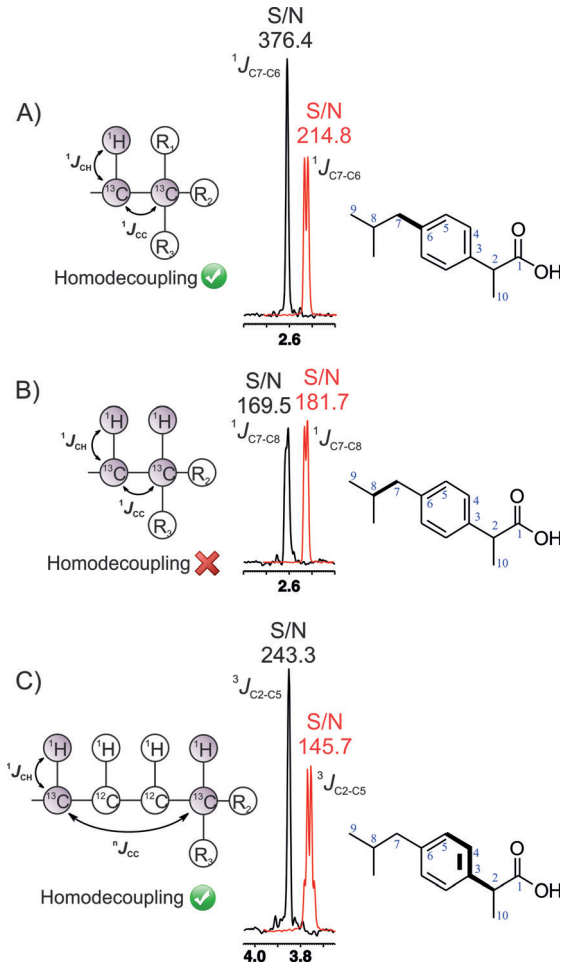

Figure 2. Experimental results of three possible types of correlation responses in 1,1- and 1,n-HD-ADEQUATE experiments using ibuprofen as a model compound (see Supporting Information, Figure S3 for experimental details). A) Correlation from a protonated carbon to an adjacent quaternary carbon via $^{1}J_{CC}$. Vicinal couplings to the detection proton are collapsed to a signlet in the 1,1-HD-ADEQUATE experiment (black trace) with a commensurate improvement in sensitivity compared to the doublet detected in the conventional 1,1-ADEQUATE experiment (red trace). Correlations of this type are not amenable to unequivocal detection in either $^{2}J,^{3}J$-HMBC[17] or H2BC[18] experiments. B) Correlations in 1,1-HD-ADEQUATE between adjacent protonated carbons are not decoupled. These correlations can, however, be established from a combination of COSY and HSQC or HSQC-TOCSY[19] spectra. C) All correlations via $^{n}J_{CC}$ homonuclear couplings in 1,n-HD-ADEQUATE spectra are beneficially collapsed to singlets with a commensurate increase in s/n. Since $^{n}J_{CC}$ correlations are the most difficult correlations to observe, the 1,n-HD-ADEQUATE provides a higher sensitivity alternative when methods such as LR-HSQMBC[6] cannot provide the required long-range correlation data.

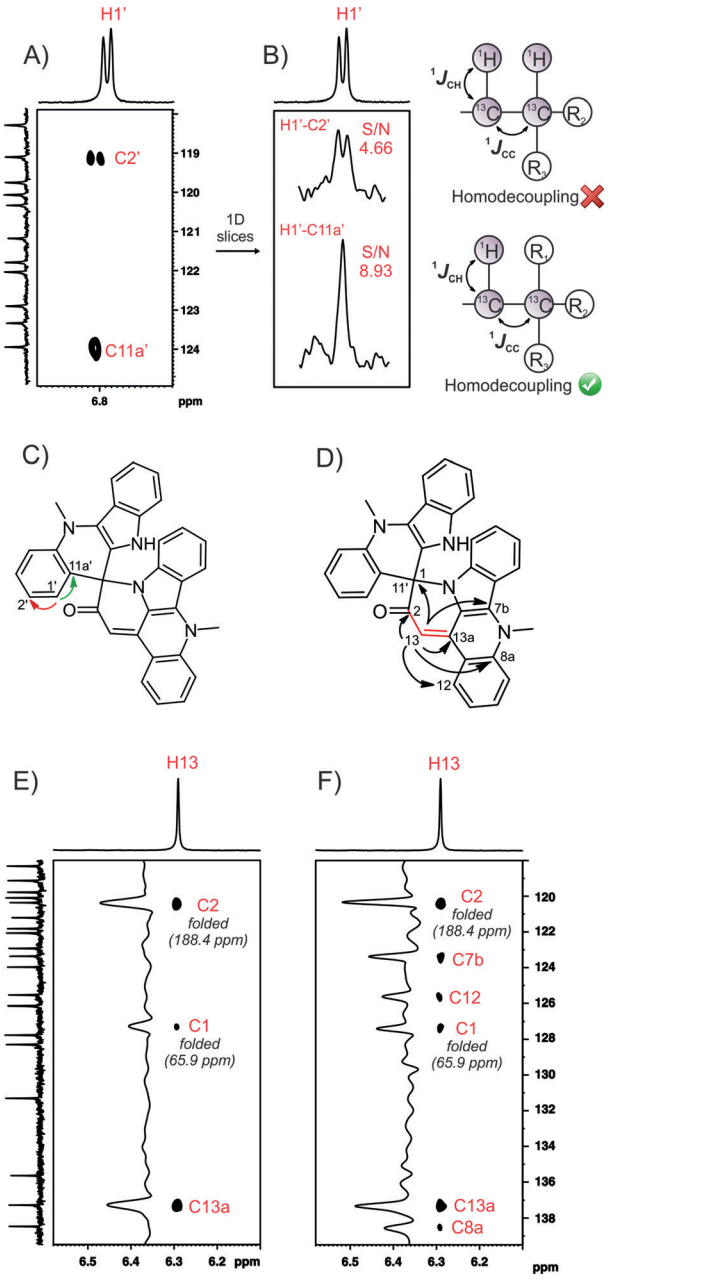

Figure 3. Experimental results obtained for 1,1- and 1,n-HD-ADEQUATE experiments performed on a 700 µg sample of cryptospirolepine (**4**) dissolved in 35 µL [D_6]DMSO. A) Segment of the 60 Hz optimized 1,1-HD-ADEQUATE spectrum of **4** showing correlations from the C1′ resonance detected via the H1′ doublet (refer to the structure in panel C) As expected from Figure 2, the C1′–C2′ correlation response is not homodecoupled. In contrast, the correlation response for the C1′–C11a′ correlation is decoupled. B) Traces for the correlation responses shown in panel (A) with better s/n for the C1′–C11a′ correlation compared to the C1′–C2′ correlation. C) Structure of **4** showing the correlations observed in panel (A). D) Structure of **4** showing the correlation results for the H13 vinyl resonance in the 1,1- and 1,n-HD-ADEQUATE spectra. E) Segment of the 60 Hz optimized 1,1-HD-ADEQUATE spectrum showing the $^{1}J_{CC}$ C13–C2 and C13–C13a correlations that allowed the revision of the structure from **1** to **4** (see Scheme 1). There is also a $^{2}J_{CC}$ correlation, C13–C1, that is observed because of the substantial size of this coupling (15.4 Hz, DFT calculated) that gives detectable intensity based on the calculated amplitude transfer curve for a 60 Hz optimized 1,1-HD-ADEQUATE spectrum. F) Corresponding segment of the 7 Hz optimized 1,n-HD-ADEQUATE spectrum showing the correlations delineated in panel (D). The carbonyl (C2) and spiro (C1/C11′) correlations were again intentionally folded. As expected, $^{1}J_{CC}$ correlations to the C2 and C13a resonances were observed in the 1,n-HD-ADEQUATE spectrum.[9]

The segments of the 1,1- and 1,n-HD-ADEQUATE spectra (see Supporting Information for plots of the full spectra) shown in Figure 3E and 3F provided the critical data necessary to successfully revise the structure of crypto-spirolepine as shown by **4**. The vinyl carbon is flanked by the carbonyl (C2) and the quaternary carbon (C13a) resonating at 188.4 (inten-

tionally folded in F_1 to improve resolution; Figure 3E) and 137.3 ppm, respectively. The numbering scheme employed is that from the original report of the structure (see Supporting Information Scheme S1 and S2).[1] There is also a folded $^2J_{CC}$ correlation to the spiro carbon, C1(C11′), observed in these data as a consequence of the calculated (DFT)[23,24] 15.4 Hz $^2J_{C13–C1}$ coupling, which will give a detectable response in a 60 Hz optimized 1,1-ADEQUATE spectrum.[8,9] Hence the [7.5.5] central core of the pentacyclic portion of the molecule, as shown by **1**, becomes a [6.6.5] moiety in **4** (see Supporting Information, Scheme S2). The problem of an unduly large $^4J_{CH}$ correlation response associated with C13a in the original structure elucidation[1] was ameliorated since the revised structure locates C13a three bonds from the aromatic proton (8.42 ppm) to which a clear correlation is observed (HMBC and LR-HSQMBC). The revised structure, **4**, also accommodates the ROE correlation between the H13 vinyl proton (6.29 ppm) and the H12 aromatic proton (8.42 ppm).

A 7 Hz optimized 1,n-HD-ADEQUATE spectrum of the sample, a segment of which is shown in Figure 3F, was also acquired (see Supporting Information), which confirmed additional homonuclear ^{13}C–^{13}C correlations consistent with the revised structure, **4**. Pertinent $^1J_{CC}$ and $^nJ_{CC}$ correlations from the 1,1- and 1,n-HD-ADEQUATE spectra are summarized on the structure shown in Figure 3D.

Given the revised structure of the alkaloid, **4**, the final ambiguity remaining was the perplexing absence of a $^2J_{CH}$ correlation from the vinyl proton to the adjacent carbonyl. This question was firmly addressed using DFT calculations, which showed the calculated $^2J_{CH}$ coupling constant to be only 0.03 Hz (see Supporting Information).[23,24] As detailed in this report, the 1,1-HD-ADEQUATE experiment provided crucial structural information that allowed the revision of the structure of cryptospirolepine as shown by **4**. The revised structure, **4**, is now consistent with the structures of the degradation products identified in 2002.[4] The extension of homonuclear decoupling to quaternary carbons in both the 1,1- and 1,n-ADEQUATE experiments affords higher sensitivity versions of these experiments that can be applied to challenging structure elucidation problems when more sensitive techniques (e.g. LR-HSQMBC[6]) fail to provide any correlation or afford correlations, the path length of which cannot be determined.[20]

Finally, the CD spectra of cryptospirolepine have no distinct features above the noise. Hence we conclude that cryptospirolepine is likely a racemic mixture. Attempts were also made to separate enantiomers using chiral HPLC methods but we have been unable to identify a column or chromatographic conditions that will separate the enantiomers of cryptospirolepine.

Experimental Section

All NMR data were acquired for a 700 μg sample of **4** dissolved in 35 μL [D_6]DMSO at 600 MHz using a Bruker AVANCE III three channel NMR spectrometer equipped with a TXI 1.7 mm MicroCryoProbe. Complete details of all acquisition and data processing parameters and acquisition times are given in the Supporting Information. Full plots of the one-dimensional proton and carbon reference spectra, the 400 ms ROESY, 60 Hz optimized 1,1-HD-ADEQUATE, and 7 Hz optimized 1,n-HD-ADEQUATE spectra are shown, as is a table of the ^1H, ^{13}C, and ^{15}N chemical shift assignments for **4**. Tables of the DFT-calculated[23,24] J_{CH} and J_{CC} coupling constants are also provided. DFT calculations were performed using the basis set and functional previously reported.[23] Excellent agreement was observed between the calculated and experimentally measured J_{CC} couplings for strychnine,[23] which suggests that the calculated J_{CC} couplings for cryptospirolepine should be reasonable. Bruker TopSpin 3.1 pulse sequence code for the HD-ADEQUATE experiments is also provided and details of the VCD/ECD work done on the sample are summarized in the Supporting Information.

Keywords: cryptolepis alkaloids · DFT calculations · HD-ADEQUATE · homodecoupling · NMR spectroscopy

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