

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

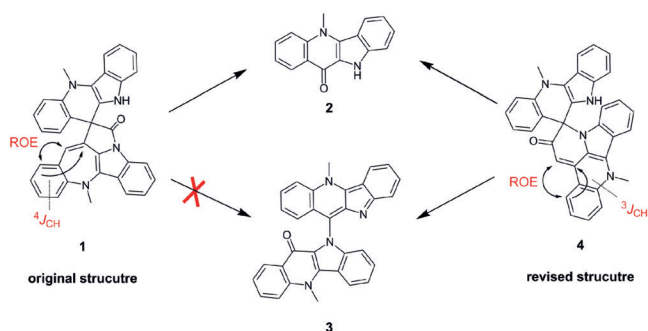
Josep Saurí, Wolfgang Bermel, Alexei V. Buevich, Edward C. Sherer, Leo A. Joyce, Maged H. M. Sharaf, Paul L. Schiff, Jr., Teodor Parella, R. Thomas Williamson,* and Gary E. Martin*

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 cryptospirolepine.

Recently, our group revisited persistent structural questions surrounding the Ghanaian 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 ¹H-¹⁵N HMBC^[2,3] experiments for natural product struc-

ture 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 ⁴J_{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 [D₆]DMSO (Scheme 1). In particular, one of these impurities, **3**, could not be mechanistically rationalized from



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.

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**.

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

[*] Dr. J. Saurí, Dr. A. V. Buevich, Dr. E. C. Sherer, Dr. L. A. Joyce, Dr. R. T. Williamson, Dr. G. E. Martin
Process & Analytical Chemistry, Merck Research Laboratories
Mailstop: 800-D133, 126 E. Scott Ave, Rahway, NJ 07065 (USA)
E-mail: gary.martin2@merck.com

Dr. W. Bermel
Bruker Biospin GmbH
Silberstreifen, 76287 Rheinstetten (Germany)

Dr. M. H. M. Sharaf
American Herbal Products Association
Silver Spring, MD 20910 (USA)

Prof. Dr. P. L. Schiff, Jr.
Department of Pharmaceutical Sciences, School of Pharmacy,
University of Pittsburgh
Pittsburgh, PA 15261 (USA)

Dr. T. Parella
Servei de Resonància Magnètica Nuclear and Departament de
Química, Universitat Autònoma de Barcelona
08193 Bellaterra, Barcelona (Spain)

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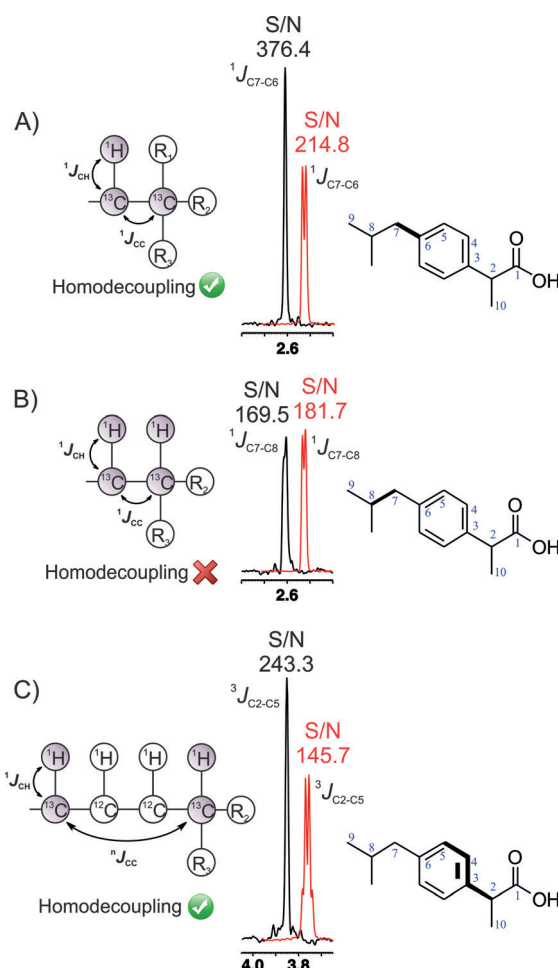


Figure 2. Experimental results of three possible types of correlation responses in 1,1- and 1,n-HD-ADEQUATE experiments using ibuprofen as a model compound (see Supporting Information, Figure S3 for experimental details). A) Correlation from a protonated carbon to an adjacent quaternary carbon via $^1J_{CC}$. Vicinal couplings to the detection proton are collapsed to a singlet 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 $^2J, ^3J$ -HMBIC^[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 $^nJ_{CC}$ homonuclear couplings in 1,n-HD-ADEQUATE spectra are beneficially collapsed to singlets with a commensurate increase in s/n. Since $^nJ_{CC}$ 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.

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-

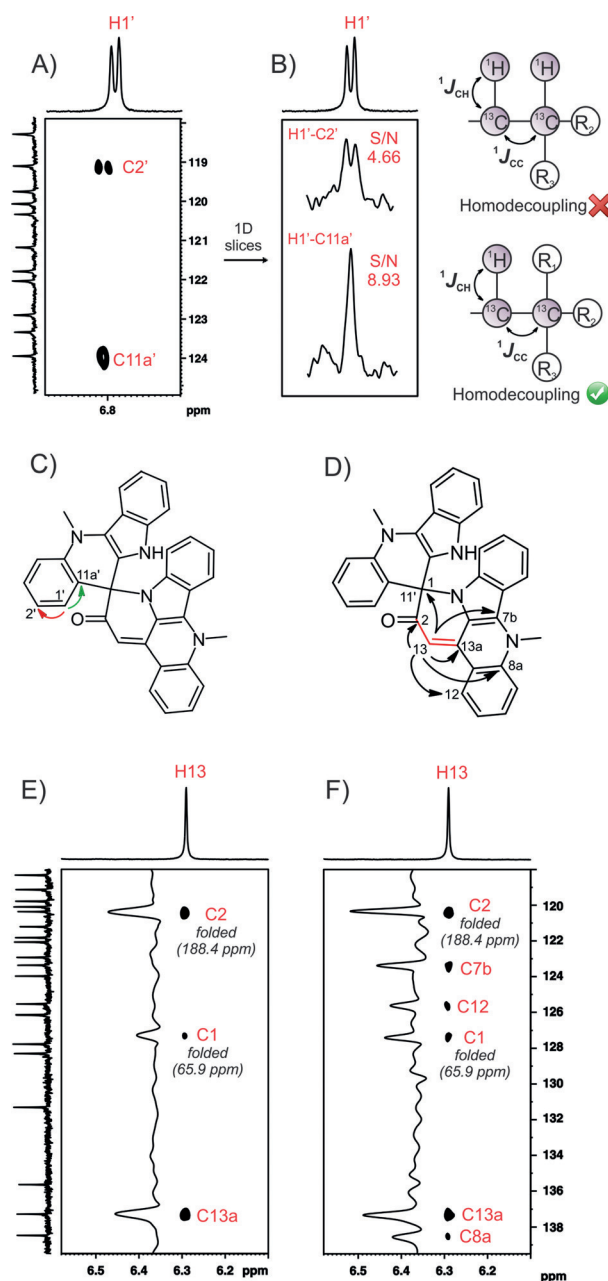


Figure 3. Experimental results obtained for 1,1- and 1,n-HD-ADEQUATE experiments performed on a 700 µg sample of cryptospirolepine (**4**) dissolved in 35 µL $[D_6]DMSO$. A) Segment of the 60 Hz optimized 1,1-HD-ADEQUATE spectrum of **4** showing correlations from the C1' resonance detected via the H1' 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 $^1J_{CC}$ C13-C2 and C13-C13a correlations that allowed the revision of the structure from **1** to **4** (see Scheme 1). There is also a $^2J_{CC}$ 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, $^1J_{CC}$ correlations to the C2 and C13a resonances were observed in the 1,n-HD-ADEQUATE spectrum.^[9]

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 μ g sample of **4** dissolved in 35 μ L $[D_6]DMSO$ at 600 MHz using a Bruker AVANCE III three channel NMR spectrometer equipped with a TXI 1.7 mm Micro-CryoProbe. 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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