Assignment of the Proton and Carbon NMR Spectra of the Indoloquinoline Alkaloid Cryptolepine

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Received February 14, 1991

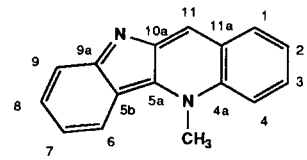
The relatively rare alkaloid cryptolepine, 5-methyl-5*H*-indolo[3,2-*b*]quinoline, was isolated from *Cryptolepis sanguinolenta* (Lindl.) Schlecter (*Asclepiadaceae*). Unequivocal proton and carbon nmr assignments in dimethyl sulfoxide are reported based on two-dimensional nmr methods, including COSY, inverse-detected direct (HMQC), and long-range (HMBC) correlations. Several of the assignments were confirmed using one-dimensional SIMBA spectra - a new, selective, one-dimensional analogue of the proton-detected long-range heteronuclear shift correlation experiment (HMBC). The SIMBA experiment was also used in an attempt to observe a two-bond coupling from the H11 proton to C10a at several optimizations.

J. Heterocyclic Chem., **28**, 1429 (1991).

Introduction.

Two-dimensional nmr, particularly the COSY experiment [2], has profoundly affected the determination of proton coupling patterns. The impact of this experiment has been particularly great in elucidating natural product structures. In instances of limited sample availability or solubility, however, investigators were frequently hampered by their inability to obtain one-bond and/or long-range heteronuclear chemical shift correlation data in reasonable amounts of time. This was a direct consequence of the relative insensitivity of heteronucleus-detected heteronuclear correlation experiments [3] (variously referred to using the acronyms HETCOR, ^{1}H-^{13}C COSY, CSCM, *etc.*) and their still less sensitive, long-range modified variants [4]. The impediments to the acquisition of heteronuclear correlation data, direct or one-bond *via* $^{1}J_{CH}$ and long-range *via* $^{n}J_{CH}$ where n = 2,3,4, have largely been obviated by the development of inverse- or proton-detected analogues of the heteronuclear correlation experiments, which are now generally referred to using the acronyms HMQC (heteronuclear multiple quantum coherence) [5] and HMBC (heteronuclear multiple bond correlation) [6]. These experiments, recently reviewed [7], allow the acquisition of one-bond or direct heteronuclear correlation spectra on sub-milligram quantities of compounds of modest (< 1000 Da) molecular weight and long-range correlation data on a few milligrams overnight.

Our objective was to apply these new techniques to obtain the total assignment of the proton and carbon nmr spectra of the alkaloid cryptolepine (**1**) (5-methyl-5*H*-indolo[3,2-*b*]quinoline) [8], which is derived from *Cryptolepis sanguinolenta* an endemic medicinal for the treatment of malaria in West Africa [9]. Our long-term goal is elucidation of structures of new indoloquinoline alkaloids. To date, there has been only one nmr study of cryptolepine, by Hufford and coworkers [10], in deuteriochloroform. We are also interested in cryptolepine and related alkaloidal constituents of *Cryptolepis sanguinolenta* and wanted to obtain chemical shift data in dimethyl sulfoxide, which is a more generally useful nmr solvent for highly substituted alkaloids.



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Results and Discussion.

Assignment of the Spectra.

The aromatic region of the 500 MHz proton nmr spectrum of **1** is shown in Figure 1. The *N*-methyl resonated at 4.922 ppm. In general, the spectrum was well resolved with only minimal multiplet overlap. The aromatic region of the carbon spectrum is shown in Figure 2. The *N*-methyl resonance was completely obscured by the DMSO multiplet but was readily observed in a DEPT spectrum to resonate at 38.9 ppm.

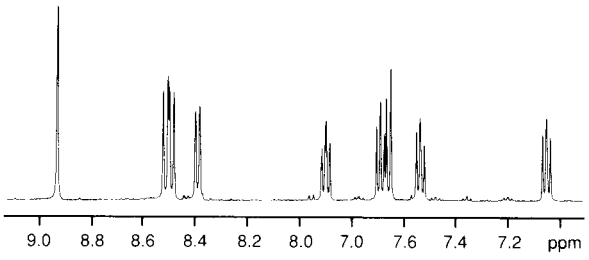


Figure 1. Aromatic Region of the Proton Spectrum of Cryptolepine in d_6-DMSO at 500 MHz.

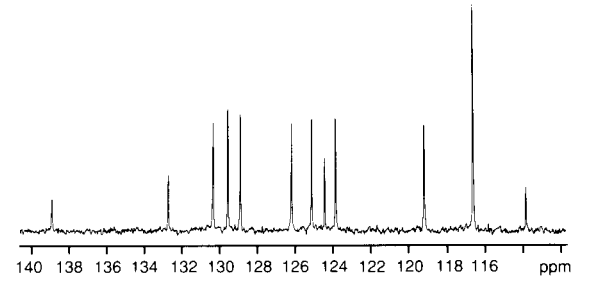


Figure 2. Aromatic Region of the Carbon Spectrum of Cryptolepine in d_6-DMSO at 75 MHz.

Within the proton spectrum, the only resonance assignable by inspection is the H11 singlet resonating furthest downfield at 8.952 ppm (see also Table 1). It was also obvious that the balance of the resonances in the aromatic region of the spectrum comprised two four-spin systems, which is consistent with the structure of **1**. The COSY

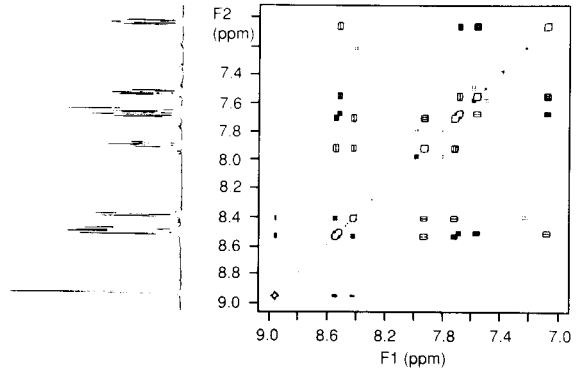
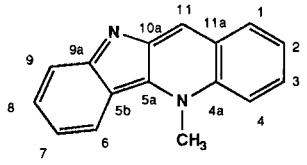


Figure 3. COSY spectrum of cryptolepine at 500 MHz acquired as 1K x 64 points with an acquisition time of 6 minutes. The data were zero-filled to 1K x 1K points, processed with sinebell weighting and symmetrized prior to plotting.

Table 1

Proton and Carbon Chemical Shift Assignments for Cryptolepine in DMSO at 500 and 75 MHz, Respectively. Coupling Constants were Determined from a Resolution Enhanced 64K Spectrum. Long-Range Heteronuclear Connectivities Were Observed in the HMBC Spectrum Optimized for 50 msec (10 Hz).



Position	Chemical Shift (ppm)			J_{HH} (Hz)	J_{CH} Pathways [a]
	^1H		^{13}C		
	DMSO	$CDCl_3$ [b]			
1	8.40	7.92	129.6	8.2, 1.4	H3, H11
2	7.69	7.46	123.9	7.9, 6.6, 0.8	H4
3	7.90	7.69	128.9	9.1, 6.8, 1.4	H1, H2 (weak)
4	8.53	7.83	116.6	9.2, 0.8	H2
4a			132.8	–	H1, H3, H11
5a			139.0	–	H11
5b			113.8	–	H7, H9, H6 (weak)
6	8.51	7.75	125.1	7.6, 0.8, 0.8	H8
7	7.05	6.76	116.6	8.6, 6.8, 1.2	H9
8	7.53	7.37	130.4	8.6, 6.6, 1.2	H6
9	7.66	7.65	119.5	8.6, 1.0, 1.0	H7, H6 (very weak)
9a			160.0	–	H6, H8
10a			144.4	–	—
11	8.95	8.45	126.2	–	H1
11a			124.4	–	H1, H2, H11
N-CH_3	4.92	4.32	38.9	–	—

[a] From the proton specified in this column to the carbon located at the indicated position in the molecular skeleton. [b] The chloroform nmr data were determined in the study reported by Ablordeppey, *et al.* [10].

spectrum (Figure 3) allowed the subgrouping and establishment of vicinal neighbor relationships within the two four-spin systems. It is plausible, extrapolating from the proton chemical shift behavior of indole, to attribute the multiplet resonating upfield at 7.045 ppm as the H-7 resonance, which provides a potential entry point into the assignment of the resonances in the indole-derived four-spin system.

The most important assignment information we acquired derives from the HMQC and HMBC spectra shown in Figures 4 and 5, respectively. The former, as noted in the introduction, establishes the direct or one-bond correlations (see Table 1). For the 5-mg sample employed in this study, an adequate HMQC spectrum can be obtained in as little as 15 minutes. The latter experiment, which was optimized for an assumed 10 Hz long-range heteronuclear coupling ($^nJ_{CH}$), provided the means of unequivocally assigning all of the proton and carbon resonances of the spectra by orienting the individual spin systems relative to one another through common couplings to identifiable points in the molecular structure.

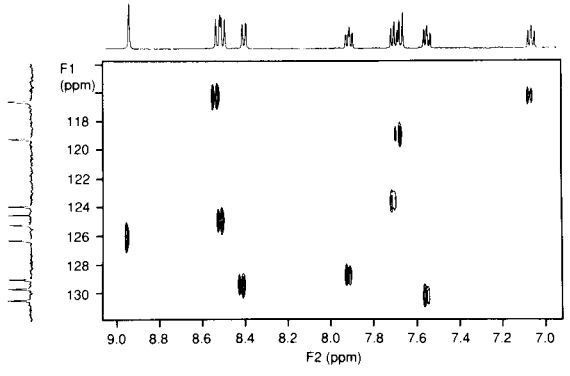


Figure 4. HMQC Spectrum of cryptolepine acquired at 500 MHz as 512 x 128 points. The data were zero-filled to 1K x 512 points and subjected to gaussian multiplication prior to both Fourier transformations. Acquisition time was 1 hour. The spectrum is flanked by high resolution proton and carbon reference spectra. The total acquisition time was 1 hour.

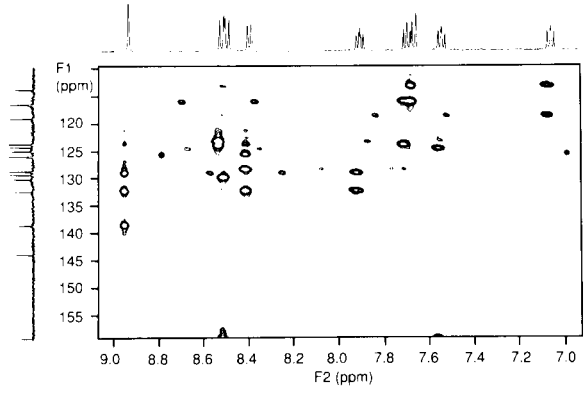
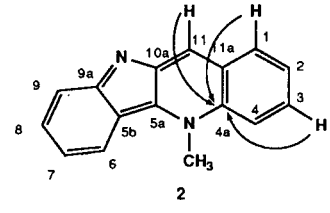


Figure 5. Full HMBC spectrum of the aromatic region of cryptolepine. The experiment was optimized for a 10 Hz long-range coupling (50 msec), the data were acquired as 512 x 256 points, zero-filled to 1K x 1 K points, subjected to phase shifted gaussian multiplication prior to the first Fourier transformation and gaussian multiplication prior to the second. The spectrum is flanked by high resolution proton and carbon refrence spectra. The total acquisition time was 8 hours.

The most convenient starting point for the assignment is the H11 singlet. As observed in the HMBC spectrum presented in Figure 5 and the expansion shown in Figure 6, connectivities are observed from H11 to the carbons resonating at 139.0, 132.8, and 129.6 ppm. The carbon resonating at 139.0 ppm showed no other connectivities and consequently may be tentatively assigned as C5a. The carbon resonating at 132.8 ppm exhibited connectivities to the "doublet" resonating at 8.397 ppm and the multiplet resonating at 7.901 ppm. From the COSY spectrum (Figure 3) we concluded that the protons at 8.397 and 7.901 ppm are meta to one another. Given this information, the only carbon that can be uniquely coupled to the three protons in question is C4a, as shown by the arrows used to denote these connectivity pathways on **2**.



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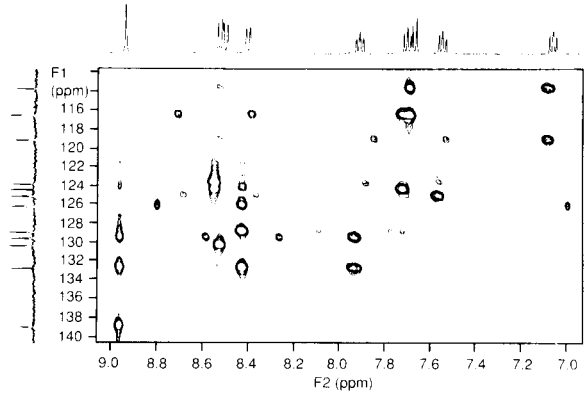


Figure 6. Expansion of the upfield portion of the aromatic region of the HMBC spectrum. The data used to generate this plot was that shown in Figure 5.

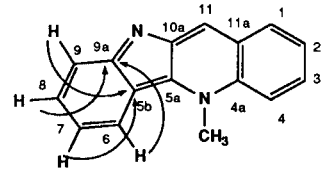
Using the connectivities just established as a point of departure, the balance of the protonated carbons, C1 through C4, may be unequivocally assigned as may be their corresponding, directly bound protons. It is also interesting to note that H1 exhibited a long-range correlation to the C11 resonance and, conversely, H11 was long-

range coupled to C1. In the absence of a common quaternary coupling partner, the two-spin systems in question could be oriented to one another from this set of coupling pathways.

Returning to the long-range correlations of H11, we have only the correlation to the carbon resonating at 139.0 ppm left to consider. The coupling possibilities that remain are $^2J_{CH}$ couplings to C10a or C11a and $^3J_{CH}$ to C5a. Of these, the coupling to C11a may be eliminated from consideration because C11a is assignable as the carbon resonating at 124.4 ppm on the basis of long-range couplings to H2 and H4. Of the two remaining coupling pathways, because $^3J_{CH}$ couplings are generally much larger than their $^2J_{CH}$ counterparts, it is logical to assign the resonance at 139.0 ppm as C5a in light of the 10 Hz optimization used to acquire the HMBC data. Although tentatively assigned as C5a, the assignment of the quaternary carbon can be unequivocally made on the basis of long-range couplings to the *N*-methyl group. While the *N*-methyl proton resonance was not included in the HMBC spectrum, this information can be and was acquired using a selective one-dimensional analogue of the HMBC experiment known as SIMBA. This aspect of the assignment is discussed in more detail below.

Assignments made thus far account for three of the six quaternary carbon resonances, leaving those at 160.0, 144.4, and 113.8 ppm to be assigned. Returning to the premise that H7, *para* to the "indole" annular nitrogen, would resonate furthest upfield at 7.045 ppm based on comparison to normal indole proton chemical shifts, we note that this proton was also long-range coupled (Figure 6) to the quaternary carbon resonating at 113.8 ppm, as was the proton resonating at 7.657 ppm. The two protons now in question were meta to one another on the basis of the COSY spectrum (Figure 2). These correlations lead to an assignment of the carbon resonating at 113.8 ppm as C5b, the chemical shift of which is also quite reasonable for its location in the structure of the molecule. This confirms the assignment of H7 at 7.045 ppm and the assignment of H9 at 7.657 ppm.

Of the two remaining quaternary carbons, the carbon resonating at 160.0 ppm was observed to long-range couple (Figure 5) to the protons resonating at 8.511 and 7.657 ppm. These responses appeared at the very edge of the F_1 spectral window; the chemical shift of this carbon was not known when the HMBC data were acquired. Rather, the carbon reference spectrum, which actually took longer, was acquired much later. Given the relationship of these protons to H7 and H9, they are assigned as H6 and H8, respectively, thereby assigning the quaternary carbon as C9a. These long-range coupling pathways are shown by **3**.



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The sole remaining quaternary carbon resonating at 144.4 ppm exhibited no long-range couplings in the 10 Hz optimized HMBC spectrum and is thus assigned as C10a. The lack of correlation responses for this carbon is not particularly surprising: the only couplings reasonably available to it is to H11 *via* $^2J_{CH}$.

Application of the Selective Inverse Multiple Bond Analysis (SIMBA) Experiment.

Before discussing the chemical shifts of cryptolepine, which merit some additional comment, we would like to discuss the application of a new one-dimensional, inverse-detected, long-range correlation experiment, SIMBA (Selective Inverse Multiple Bond Analysis) [11]. Initially, the assignment of H6 through H9 was based on the assumption that H7 would resonate furthest upfield of the nine aromatic protons, which is reasonable. If this assignment were incorrect, however, in this case it would have been possible to correct it on the basis of the chemical shift of C5b, which could not be mistaken for either the C9a or C10a resonances, both of which are nitrogen-bearing quaternary carbons. If these assignments were equivocal, it would have been necessary to resort to additional experimentation. Several alternatives exist. The simplest in the present case is probably a one-dimensional nOe from the *N*-methyl to establish the identity of H4 and H6.

A solution with a broader range of utility is afforded by the acquisition of additional long-range heteronuclear correlation data. Two coupling pathways are of interest in this regard. A three-bond coupling of the *N*-methyl group to C5a, if the assignments of C5a and C10a were in question, would be useful. A two-bond coupling correlating H11 to C10a would also be helpful. The former was not included in the original F_2 frequency range of the HMBC spectrum but could have been. The latter would require the reoptimization of the delays for something less than the 10-Hz optimization used, necessitating the acquisition of an additional HMBC spectrum that clearly represents a significant and potentially prohibitive investment in spectrometer time. Unfortunately, there is also no guarantee that the expenditure of time required for the acquisition of a second HMBC spectrum would lead to the observation of the long-range coupling response sought. These constraints are circumvented, however, by the recently described one-dimensional analogue of the HBMC experiment known as SIMBA [11]. This experiment, in practical terms, is perhaps best compared to the INAPT heteronucleus-detected experiment described several years ago by Bax and coworkers [12]. The pulse sequence for the

SIMBA experiment is shown in Figure 7. Quite simply, the SIMBA experiment differs from the more familiar two-dimensional HMBC experiment only in that the final carbon pulse is applied selectively, and the evolution time is never incremented, yielding a one-dimensional proton spectrum in which the only resonances observed are those that long-range coupled to the selectively pulsed carbon. It should also be noted that the proton resonances observed in SIMBA traces will appear with the heteronuclear long-range coupling.

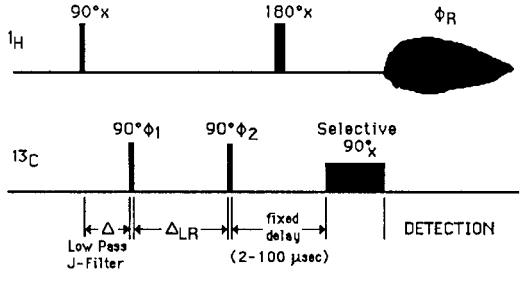


Figure 7. SIMBA pulse sequence [11]. The experiment is a straightforward modification of the HMBC experiment of Bax and Summers [6]. Details of the optimization of the delays and the calibration of the soft pulse used were given previously [11]. Traces shown in Figure 8 were optimized for 63 msec and were acquired as 8K points x 128 transients giving an acquisition time of 5 min/trace or "slice."

Application of the SIMBA experiment to cryptolepine (**1**) gave some interesting results. Carbons that were selectively pulsed included: C11a (124.4 ppm), C4a (132.8 ppm), C5a (139.0 ppm), C10a (144.4 ppm), and C9a (160.0 ppm). The individual traces shown in Figure 8 plotted above a high resolution proton reference spectrum represent an accumulation time of 5 min/trace with the experiment optimized for 63 msec (8 Hz).

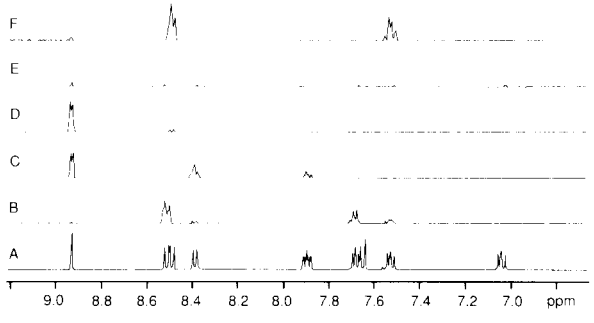


Figure 8. SIMBA traces recorded for quaternary carbons of cryptolepine plotted above a high resolution proton reference spectrum of the aromatic region. A) high resolution reference spectrum; B) trace resulting from the application of the selective pulse to C11a; C) trace resulting from the application of the selective pulse to C4a; D) trace resulting from the application of the selective pulse to C5a; E) trace resulting from the application of the selective pulse to C10a; F) trace resulting from the application of the selective pulse to C9a.

The results of the first of these experiments exactly parallel the data obtained from the HMBC spectrum. As expected, coupling pathways for C11a to H2 and H4 were observed, giving strong responses in Trace B. A much weaker response was observed to H1. The response to H8 is an artifact arising from the partial excitation of C6 (125.1 ppm) by the selective pulse at the end of the SIMBA applied to C11a at 124.4 ppm. The C6 resonance, of course, was long-range coupled to H8. Selectively pulsing C4a gave the SIMBA spectrum shown in Trace C of Figure 8. Strong responses were observed to H1 and H11 with a somewhat weaker response to H3. As shown in Trace B of Figure 9, C4a also gave the expected connectivity to the *N*-methyl group resonating at 4.922 ppm. The SIMBA spectrum of C5a is shown in Trace D of Figure 8 and Trace C of Figure 9 and showed connectivities to H11 and the *N*-methyl, respectively. Unfortunately, when optimized for 63 msec, C10a, when selectively pulsed in a SIMBA experiment, failed to give any responses (Trace E of Figure 8). A similar situation prevailed when the SIMBA experiment was optimized for 128 msec. Obviously, it is far more productive to make this determination from a 5 min SIMBA experiment rather than consume eight to ten hours of instrument time to generate a second HMBC spectrum, only to find that this experiment did not give the connectivity response sought. Finally, a SIMBA spectrum performed on the C9a resonance gave, as expected, responses to the H6 and H8 resonances.

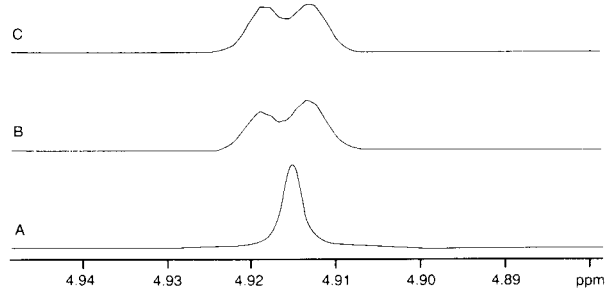


Figure 9. Aliphatic portions of the SIMBA spectra recorded for C4a and C5a plotted above a proton reference spectrum of the *N*-methyl group. A) Segment from the high resolution proton spectrum showing the *N*-methyl singlet; B) portion of the SIMBA spectrum for C4a showing the appearance of the response to the *N*-methyl group as a 2.6-Hz doublet; C) portion of the SIMBA spectrum of C5a showing the response to the *N*-methyl group as a 2.6-Hz doublet.

Another useful feature of the SIMBA experiment, alluded to above, is its capacity to allow the unequivocal measurement of the long-range heteronuclear coupling constant(s). Although this will constitute superfluous information in most structure elucidation situations, the method can be invaluable when it is necessary to measure a heteronuclear coupling and when there are potentially many

such couplings to a given carbon. The appearance of this information is most easily perceived from the connectivities of C4a and C5a to the H11 and *N*-methyl singlets. In Figure 8 Trace C and Figure 9 Trace B, C4a exhibits couplings of 4.7 Hz to H11 and 2.6 Hz to the *N*-methyl, respectively. In similar fashion, from Figure 8 Trace D and Figure 9 Trace C, respectively, we note that C5a exhibits couplings of 5.1 Hz to H11 and 2.6 Hz to the *N*-methyl.

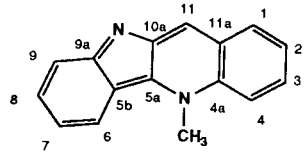
Chemical Shift Behavior of Cryptolepine.

It is interesting to note the location of the double bonds in the structure of cryptolepine (**1**) as drawn. The compound's deep violet color in solution is indicative of the extended conjugation and the fixation of the double bonds. Thus, the benzene ring of the indole portion of the molecule, while still aromatic in that it does contain six shared electrons, has two double bonds which are shared into the five-membered ring. One of these, C9a, resonated considerably downfield of what was expected when the HMBC data were acquired. The chemical shift of this carbon at 160.0 ppm in very substantially downfield of the corresponding chemical shift for the carbon at this position in indole which resonates at 135.5 ppm [13]. In similar fashion, the C5b resonance at 113.8 ppm is shifted considerably upfield of the corresponding position of indole itself, which resonates at 127.6 ppm. From the localization of the double bonds implied by several of the chemical shifts and the color, the distribution of electron density in cryptolepine is clearly not that of a simple polynuclear heteroaromatic compound. In part, it is interesting to speculate that the electronic properties of this molecule may have some role in the activity of this compound as an antimalarial when used in this capacity by the peoples of West Africa. This, however, is conjectural and remains to be explored further as potentially more active constituents of *Cryptolepis sanguinolenta* are isolated, their structures determined, and the biological activity evaluated.

It will also be noted from Table 1 that cryptolepine exhibits a considerable variation in proton chemical shift when solvent is changed from DMSO, which was used in this study, to chloroform, as in the study reported by Ablordeppey and coworkers [10]. Although none of the proton chemical shifts crossed in changing solvents, care must clearly be exercised when comparisons are made of data acquired in different nmr solvents.

Conclusions.

The proton and carbon nmr spectra of the indoloquinoline alkaloid cryptolepine (**1**) have been totally and unequivocally assigned. The two-dimensional data acquired in this study were supplemented by long-range heteronuclear connectivity information from a new one-dimensional analogue of the HMBC experiment known by the acronym SIMBA. The flexibility of the SIMBA experiment allows the acquisition of only limited long-range connectivities for a few carbons, the exploration of other delays to obtain long-range responses that were not observed in an HMBC experiment, or connectivity information to protons or carbons that it might be desireable to exclude from an HMBC experiment to limit spectral widths and digitization requirements.



EXPERIMENTAL

The sample of cryptolepine employed in this study, 5.1 g, was isolated from *Cryptolepis sanguinolenta* as described previously [9]. The sample was dissolved in 0.8 ml of 100% hexadeuteriodimethyl sulfoxide (Merck) and filtered through glass wool into a 5-mm nmr tube. Proton detected nmr experiments were performed using a Varian VXR 500-S spectrometer operating at a frequency of 499.843 MHz and equipped with a 5-mm Z-Spec inverse detection probe supplied by Nalorac Cryogenics Corporation. Carbon spectra were acquired using a Varian VXR 300-S spectrometer operating at a frequency of 75.429 MHz and equipped with a 5-mm switchable probe.

The HMQC data were acquired using the sequence of Bax and Subramanian [5] as 128 x 512 point fids with an acquisition time of 0.239 sec/transient. The data were zero-filled to 1024 x 512 points during processing. The 90° proton pulse width was 11.9 μsec; the ^{13}C 90° pulse from the decoupler was 11 μsec with an effective decoupling field, $\gamma H_2/2\pi = 19.4$ KHz. A total of 8 transients were acquired per t_1 increment. Spectral widths were 1072 Hz in F_2 and 6285 Hz in F_1. The null delay was set to 300 msec and the interpulse delay was 1.2 sec. Total acquisition time for the data was 30 minutes.

The long-range HMBC data were acquired using the pulse sequence of Bax and Summers [6]. Pulse widths were identical to those employed for the HMQC data. The data were acquired with the delay for long-range correlation optimized for 50 msec (10 Hz). The low-pass J-filter was optimized for 165 Hz. The data were acquired as 256 x 512 point fids and were zero-filled to 2048 x 1024 points during processing. A total of 64 transients were acquired per t_1 increment. Total acquisition time was 8.5 hours.

The SIMBA spectra were acquired using the pulse sequence of Crouch and Martin [11] shown in Figure 7. The 90° ^1H and ^{13}C pulses were identical to those employed for the HMQC/HMBC spectra. The selective 90° ^{13}C pulse employed was 7 msec followed by a 5-msec delay prior to data acquisition. The selective pulse was calibrated using a protonated carbon resonance in the cryptolepine spectrum. When employed in this fashion, the SIMBA experiment gives a spectral result comparable to the SELINCOR experiment described by Berger [14]. A rough approximation of the pulse calibration was made for a hard 90° ^{13}C pulse attenuated from 60-to 6 dB with a 6-dB attenuator on the power input to the 100-watt ENI amplifier used in the VXR-500S

on which this work was performed. The selective pulse was recalibrated when the instrument was configured to perform in the inverse mode by the acquisition of a series of ^{13}C-coupled SELINCOR spectra. The pulse width employed was chosen on the basis of observed signal intensity and suppression of residual ^{1}H-^{12}C signal with more emphasis placed on the latter. Practically, the absolute tip angle of the selective pulse is not critical, pulse widths between 60-120° will give usable performance. The refocusing delay was optimized by arraying the duration of the refocusing delay and selecting the interval that gave the best suppression of unwanted signal. The SIMBA spectra were digitized with 8K data points giving an acquisition time of 0.924 sec per transient. Total time per trace in the SIMBA experiment was approximately 5 minutes.

Acknowledgments.

The authors kindly acknowledge the assistance of E. C. O. Ansah for collection of plant material and Ehene-Ofori for assistance in the extraction process.

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Note Added in Proof.

We would like to note the appearance of a paper describing a pulse sequence and results similar to those obtained with the SIMBA pulse sequence used in this work. M. A. Keniry and G. A. Poulton, *Magn. Reson. Chem.*, **29**, 46 (1991), described a "soft" heteronuclear multiple bond technique and the results obtained by the application of this method to quaternary carbon resonances of the fungal metabolite lambertellin.