Total Assignment of the Proton and Carbon NMR Spectra of the Alkaloid Quindoline - Utilization of HMQC-TOCSY to Indirectly Establish Protonated Carbon-Protonated Carbon Connectivities

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The reisolation of the indoloquinoline alkaloid quindoline (also known as norcryptolepine) from *Cryptolepis sanguinolenta* is reported. The structure was unequivocally confirmed by two-dimensional nmr methods; the proton and carbon spectra were assigned for the first time. Because of congestion in the proton spectrum HMQC-TOCSY was used as an alternative to the more familiar COSY experiment. In addition to establishing proton-proton connectivities, HMQC-TOCSY affords the added benefit of providing, in an indirect sense, connectivity information between protonated carbons.

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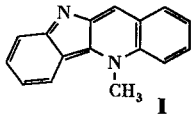
Introduction.

Several recent reports have focused on the structures and nmr spectroscopy of the alkaloidal constituents of the West African medicinal plant *Cryptolepis sanguinolenta* (Lindl.) Schlecter (*Asclepiadaceae*). The isolation of cryptolepine (**1**) from *C. sanguinolenta* was recently reported by Ablordeppy *et al.* [1] and our group [2]. Additional work on the alkaloidal fraction of an extract of *C. sanguinolenta* has resulted in the reisolation of a second indoloquinoline alkaloid, quindoline (also known as norcryptolepine) (**2**), which has never been examined by nmr, muchless two-dimensional nmr techniques. Quindoline was first described by Fichter and Boehringer in 1906 as a synthetic product [3]. The alkaloid was not isolated from a plant source until 1978, when Dwuma-Badu *et al.* described the compound as a metabolite of *C. sanguinolenta* [4].



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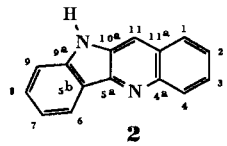

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In light of the question of double bond locationization which arose from our previous nmr study of cryptolepine (**1**) [2] and since no nmr data for quindoline have been reported, we were interested in obtaining a total proton and carbon nmr resonance assignment for this relatively rare alkaloid.

Results and Discussion.

The proton nmr spectrum of quindoline (**2**) at 500 MHz was still relatively congested; the carbon spectrum, in contrast, was quite well dispersed. Using the inverse-detected HMQC pulse sequence of Bax and Subramanian [5], the heteronuclear correlation spectrum shown in Figure 1 was obtained on a 10 mg sample of **2** in less than 30 minutes. Clearly the most problematical region of the proton spectrum was near 7.6 ppm with three of the proton resonances vicinally coupled to two neighbors and one other resonance partially overlapped (see Figure 1). Establishing the proton-proton connectivities in this regionusing a COSY spectrum could lead to correlations which are difficult to track unequivocally. These difficulties are obviated, however, through the use of HMQC-TOCSY spectra, which allow proton-proton connectivity information to be sorted with the generally much higher dispersion afforded by the ^{13}C spectrum.

HMQC-TOCSY spectra of quindoline were acquired using a modification of the sequence originally described by Lerner and Bax [6]. Rather than delaying the onset of broadband heteronucleus decoupling to cancel the direct responses, we find it advantageous to initiate broadband heteronucleus decoupling at the beginning of the acquisition period, thereby retaining the direct responses as described in a recent review [7]. Mixing times employed ranged from 12 to 48 msec. To illustrate the identification of the protonated carbon constituents of one of the two four-spin systems of quindoline, the proton doublet resonating at 8.35 ppm was utilized. The data presented in

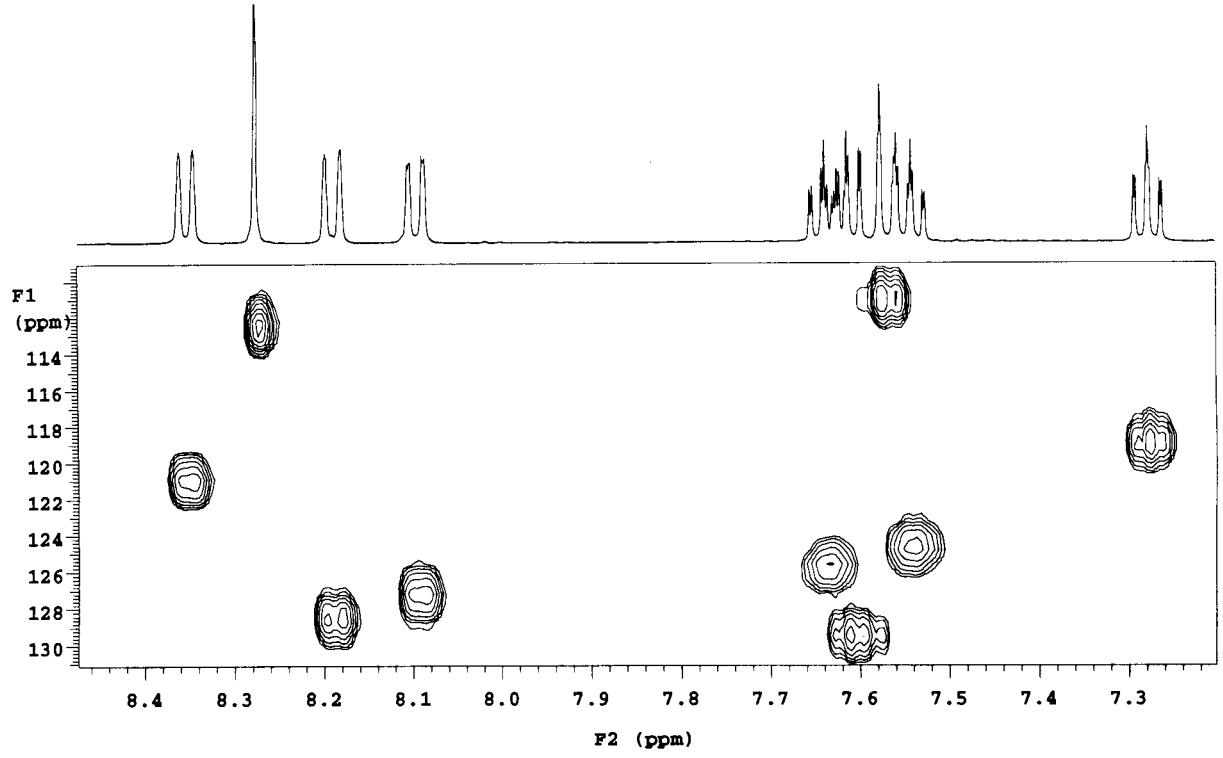


Figure 1. HMQC spectrum of quindoline in DMSO recorded at 500 MHz. The contour plot is flanked by a high-resolution proton reference spectrum.

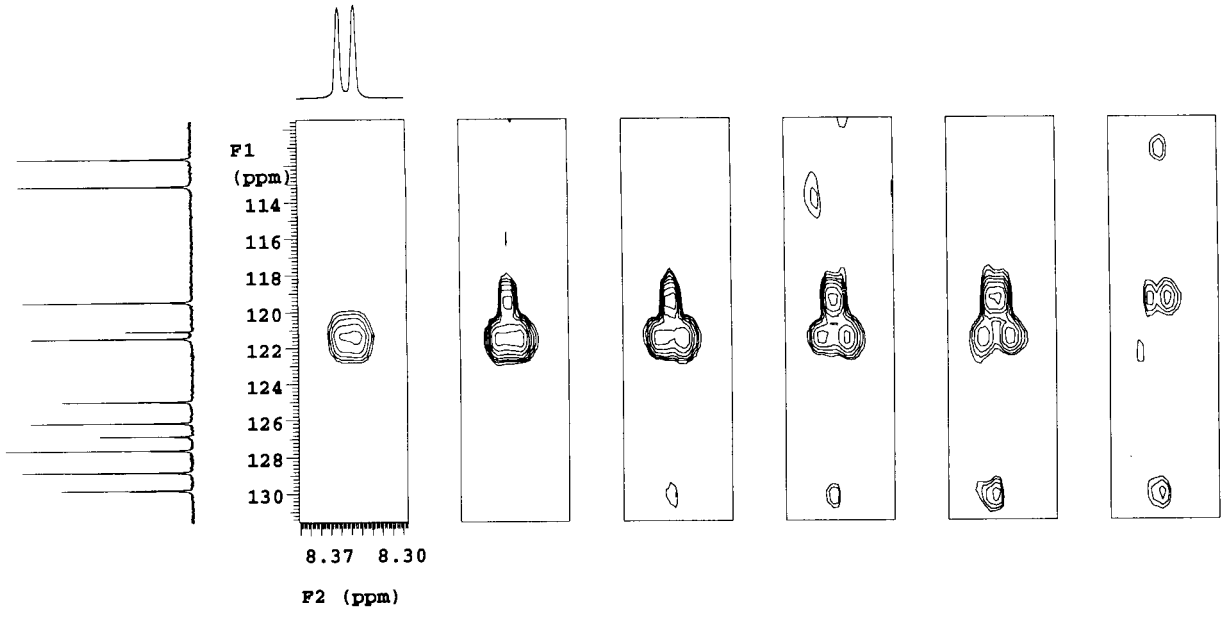
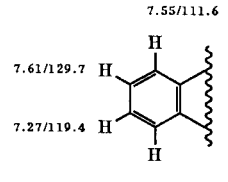


Figure 2. HMQC-TOCSY/HMQC spectral composite for correlations to the proton resonating at 8.35 ppm. The HMQC spectral segment is shown to the left; corresponding regions through the F_1 frequency domain from the series of HMQC-TOCSY spectra are shown to the right beginning with the 12 msec experiment.

Figure 2 begin with an F_1 segment taken from the HMQC spectrum (Figure 1), which shows the location of the direct response. Successive segments from the left to right illustrate the corresponding regions of HMQC-TOCSY spectra with mixing times of 12, 18, 24, 30, and 48 msec, respectively. As is readily noted in the slice from the 12 msec spectrum, a response is observed in F_1 corresponding to the carbon resonating at 119.1 ppm. In the 18 msec panel, a new response is observed at the F_1 frequency corresponding to the carbon resonating at 129.5 ppm. The information available at this point persists, except for changes in response intensities, through the 24 and 30 msec spectra. Finally, in the 48 msec spectrum, a response is observed at the F_1 frequency of the carbon resonating at 111.0 ppm. It should also be noted that at this mixing time, the direct response at 121.4 ppm has now been lost.

The series of operations just described establishes, in an indirect fashion, connectivities between contiguous protonated carbon resonances. This should not, however, be taken to infer that this process utilizes any form of carbon-carbon coupling or multiple quantum coherence as in the INADEQUATE [8] experiment. All of the information derived from the series of HMQC-TOCSY spectra is transmitted *via* proton-proton scalar coupling pathways. In the case of the 12 msec HMQC-TOCSY spectrum, which is presented in Figure 3 as the bottom panel, the direct response is observed at 121.4/8.35 ppm. A vicinal relay response is observed at the proton chemical shift of the vicinal neighbor proton at 7.28 ppm. Conversely, the 119.4/7.28 ppm direct response exhibits a relayed response to the proton resonating at 8.35 ppm (also in the bottom panel of Figure 3). Thus, the response observed in the 12 msec panel of Figure 2 arises from relay of magnetization from the 7.28 ppm proton to the 8.35 ppm proton which is observed at the F_1 frequency of the carbon bound to the 7.28 ppm proton.

Practically, the operations described above in conjunction with the data contained in Figure 2 allow the assembly of a structural fragment shown by **3**.



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Although complicated somewhat by the strong coupling of two of the protons and the similarity of the carbon chemical shifts, the other four-spin system can be assem-

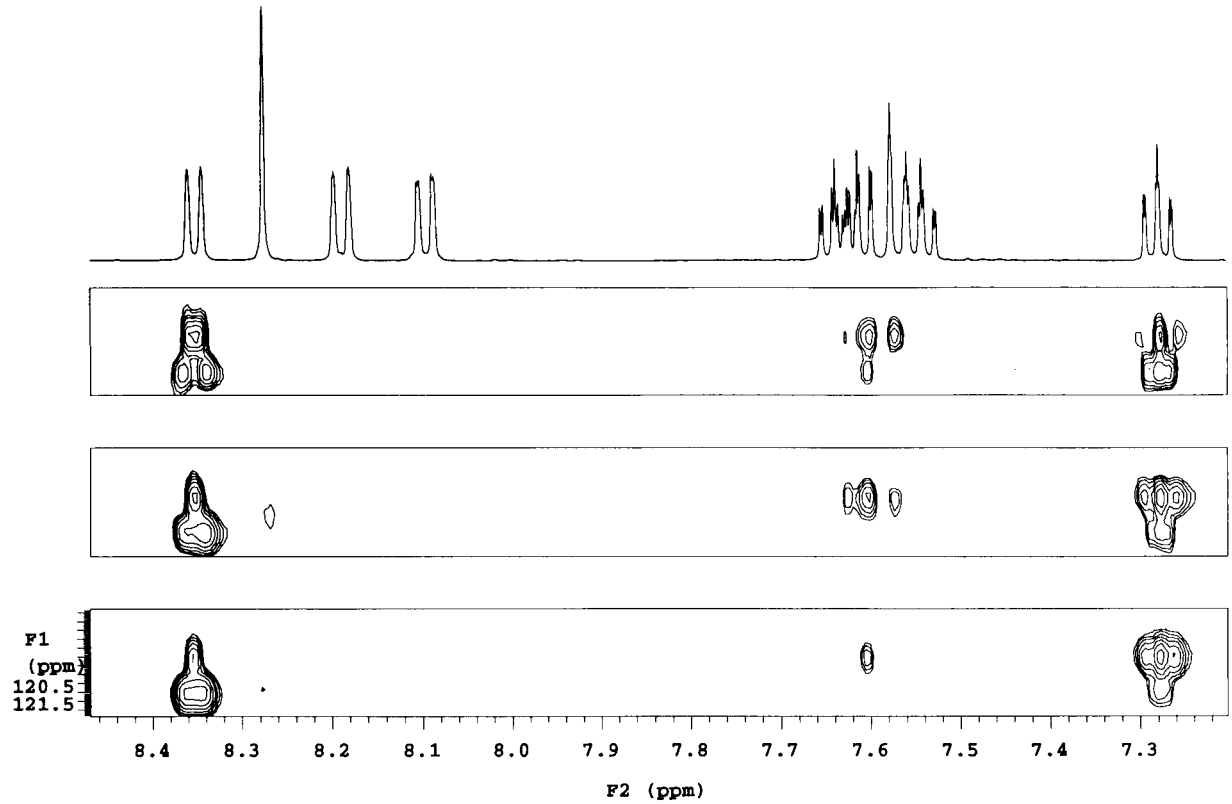
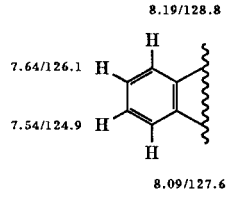


Figure 3. HMQC-TOCSY spectral regions through the F_1 frequency domain for the proton resonating at 8.35 ppm. The slices shown are taken from spectra acquired with mixing times of 12 (bottom), 18 (middle) and 30 (top) msec. A high-resolution proton spectrum is plotted across the top.

bled in similar fashion. The proton resonating at 8.19 ppm is correlated to the proton resonating at 7.64. The proton resonating at 8.09 ppm is correlated to its vicinal neighbor which resonates at 7.54 ppm. The 24 msec HMQC-TOCSY spectrum defines the correlation between the 7.54 and 7.64 ppm confirming what is already the obvious connectivity of the remaining four-spin system, as shown by **4**.

The task remaining is to connect the two four-spin systems to the isolated spin *via* the six quaternary carbon resonances. This task was conveniently accomplished using an HMBC spectrum optimized for a 10 Hz (50 msec) long-range coupling pathway. The HMBC spectrum of quindoline is shown in Figure 4.



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Beginning from the 8.27 ppm singlet, couplings were observed to the carbons resonating at 145.7, 143.4, and 127.4 ppm. The first two resonances of this group were quaternary carbon resonances, the last was the protonated carbon bound directly to the proton resonating at 8.09 ppm. The proton resonating at 8.09 ppm was also long-range coupled to the quaternary carbon resonating at 143.4 ppm, as was the proton meta to it at 7.64 ppm. Hence, the carbon resonating at 143.4 ppm is assigned as C4a. The other quaternary carbon resonating at 145.7 ppm must thus be the C5a resonance. Further confirmation of the assignment of C5a is afforded by the long-range coupling of the 145.7 ppm resonance to the NH resonance at 11.4 ppm. The latter assignment is also confirmed by the weak three-bond connectivity from the proton resonating at 8.35 ppm which must be the H7 resonance. A summary of all of the long-range connectivities to the quaternary carbons is shown by **5**.

The assignment of the other quaternary carbon resonances was quite straight-forward. The carbon resonating at 144.1 is assigned as C9a and is long-range coupled with

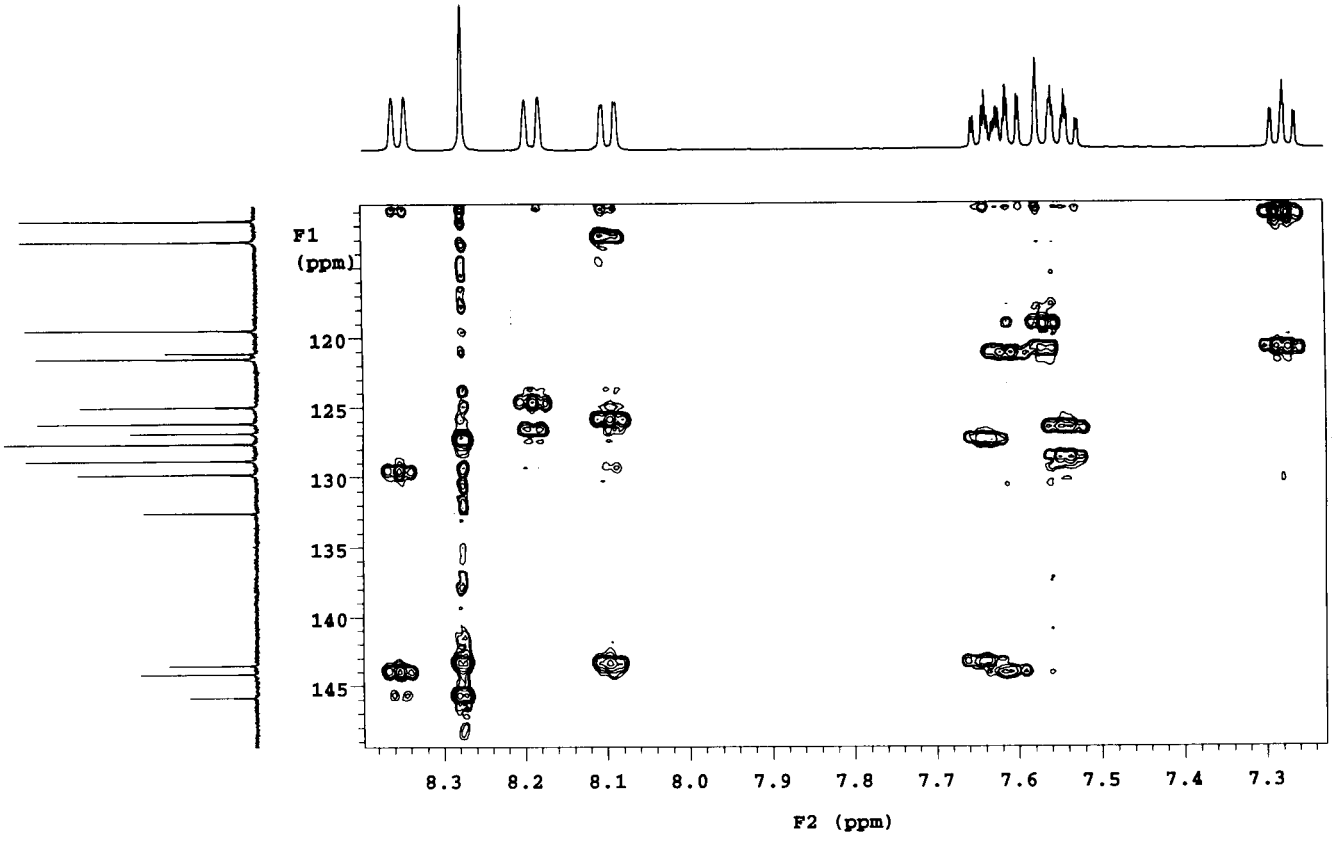
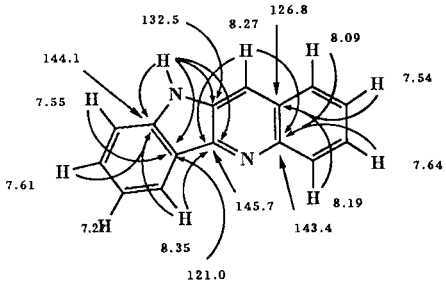


Figure 4. HMBC spectrum of quindoline optimized for an assumed long-range coupling of 10 Hz. The spectrum is flanked by high-resolution proton and carbon reference spectra.

the protons at 8.35 and 7.61 ppm, which are assigned as H6 and H8, respectively, as well as the NH resonance at 11.4 ppm. The carbon resonating at 121.0 ppm is long-range coupled to protons resonating at 7.55 and 7.27 ppm (H7 and H9, respectively) and may thus be assigned as C5b. A coupling to the C5b resonance from the NH resonance at 11.4 ppm is also observed. The carbon resonating at 126.8 ppm is long-range coupled to protons resonating at 7.54 and 8.19 ppm (H2 and H4, respectively) and may thus be assigned as C4a. Finally, the quaternary carbon resonating at 132.5 ppm exhibits only a sole long-range coupling in the HMBC spectrum, that to the NH proton. This is not surprising since the only other potential long-range response to this carbon is the two-bond coupling to the H11 resonance which is generally weak or not observed.



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Conclusions.

The structure of a compound tentatively identified as quindoline on the basis of its physical constants has been unequivocally confirmed through the use of inverse-detected two-dimensional nmr techniques. Total assignments of the proton and carbon nmr spectra have been made. The chemical shift of the C9a resonance of quindoline, significantly upfield of the corresponding resonance of cryptolepine [2], tends to support the contention that the electrons that would comprise one of the double bonds of the indole-derived aromatic ring are fixed exo to the ring in the imine bond C9a-N10 and C5a-C5b as shown by **1**. Finally, a convenient method to establish connectivities between contiguous protonated carbons which utilizes a series of high-sensitivity HMQC-TOCSY spectra has been illustrated [7].

EXPERIMENTAL

Isolation.

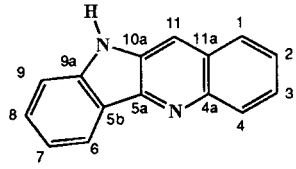
The reisolation of quindoline from an air-dried, ground sample of *C. sanguinolenta* was conducted in the manner described in the earlier work of Dwuma-Badu *et al.* [4].

NMR Spectroscopy.

All of the nmr experiments described in this work were performed on a 10 mg sample of quindoline dissolved in 0.8 ml of 99.96% d_6-DMSO. All experiments were performed using a Varian VXR-500S spectrometer operating at 499.843 MHz and equipped with a Z-Spec ID-500 probe obtained from Nalorac Cryogenics Corp., Martinez, CA. The HMQC spectrum was recorded using the pulse sequence of Bax and Subramanian [5] as 64 x 512 data points. The data were processed using a Gaussian multiplication with zero filling to give a final data matrix comprised of 256 x 1024 points. Total acquisition time for the HMQC data was 26 minutes. The HMQC-TOCSY spectra acquired utilized the pulse sequence of Lerner and Bax [6] modified as described in the review of Martin and Crouch [7]. The spectra were acquired as 48 x 512 data points with mixing times ranging from 12 to 48 msec. The data were processed using a Gaussian multiplication and were zero filled to afford a final data matrix consisting of 256 x 1024 points. Total acquisition time/HMQC-TOCSY spectrum was 20 minutes. Finally, an HMBC spectrum was obtained optimized for a long-range coupling of 10 Hz (50 msec) was acquired as 128 x 768 data points. The interpulse delay was increased to 3 seconds for this experiment from the 1.2 seconds used for all other spectra described above. The data were processed using multiplication by a phase shifted Gaussian and a Gaussian and were zero filled to give a final data matrix consisting of 512 x 2048 points. Total acquisition time for the HMBC spectrum was 3.75 hours.

Table 1

Proton and Carbon NMR Resonance Assignments for Quindoline.



Position	δ^1H (ppm)	δ^{13}C (ppm)
5a	-	145.7
9a	-	144.1
4a	-	143.4
10a	-	132.5
8	7.61	129.8
4	8.19	128.8
1	8.09	127.6
11a	-	126.8
3	7.64	126.1
2	7.54	124.9
6	8.35	121.4
5b	-	121.0
7	7.27	119.4
11	8.27	113.1
9	7.55	111.58
NH	11.4	-

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