Elucidation of the Structure of Quindolinone, a Minor Alkaloid of *Cryptolepis sanguinolenta*: Submilligram ^{1}H-^{13}C and ^{1}H-^{15}N Heteronuclear Shift Correlation Experiments Using Micro Inverse-Detection

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Elucidation of minor natural product structures has been significantly augmented by inverse-detection; further improvement has been afforded by the development of micro inverse-detection probes. We report here the elucidation of the structure of a new alkaloid, quindolinone (5*H*,10*H*-indolo[3,2-*b*]quinolin-11-one), from the West African plant *Cryptolepis sanguinolenta*. All nmr data for this minor, preparative hplc-isolated alkaloid, including ^{1}H-^{15}N one-bond heteronuclear shift correlation (HMQC) data, were recorded on an 800 μg sample of the alkaloid dissolved in 140 μl of 100% d_6-DMSO using a 400 MHz spectrometer.

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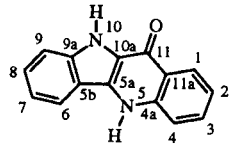
Molecular structure elucidation has been greatly augmented by the advent of inverse-detected heteronuclear shift correlation techniques [1-3]. Still, when dealing with a total unknown, progress toward the completion of the structure determination can be hampered by the acquisition of a ^{13}C reference spectrum of acceptable quality. The development of micro dual and heteronuclear Nanoprobes™ have, however, made it feasible to acquire spectra on samples as small as 0.1 μmole on a 500 MHz instrument in periods of time ranging from overnight to over a weekend. The enhancement in inverse-detected heteronuclear shift correlation experiments when using micro inverse-detection probes has been documented [3-5]. More recently, we have also reported data comparing carbon-optimized micro dual and heteronuclear nanoprobes for the acquisition of ^{13}C reference spectra [6,7]. Thus, probe hardware is now available that makes it possible to conveniently elucidate the structures of minor alkaloids at the submilligram levels in quite reasonable periods of time.

We now wish to report the elucidation of the structure of quindolinone (**1**) (5*H*,10*H*-indolo[3,2-*b*]quindolin-11-one), a minor alkaloid isolated from the indigenous West African medicinal plant *Cryptolepis sanguinolenta*. The structure was elucidated using a total sample of 800 μg dissolved in 140 μl of 99.96% d_6-DMSO (Cambridge Isotope Laboratories). All ^{1}H, ^{13}C, and ^{1}H-^{13}C HMQC [8] and HMBC [9] data were acquired using a Varian Unity 400 spectrometer equipped with a Nalorac Z•SPEC® MD-400 microdual probe; the ^{1}H-^{15}N HMQC spectrum was acquired on the same instrument using a Nalorac Z•SPEC MID-400 micro inverse-detection probe.



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The ^{1}H reference spectrum is shown in Figure 1. The spectrum is comprised of resonances consistent with two four-spin systems and two broadened, one proton resonances downfield at 12.43 and 11.66 ppm. The latter were typical of NH resonances in the spectra of quindoline [10] and cryptospirolepine [11]. Although readily available, the acquisition of a COSY spectrum was obviated by the long-range heteronuclear couplings obtained from an HMBC spectrum, which allows the indirect identification and sequencing of the proton spin systems.

Normally, at the submilligram level, the greatest impediment to structure elucidation may be the acquisi-

tion of a ^{13}C reference spectrum. In many instances the indirect determination of ^{13}C chemical shifts from inverse-detected 2D data will suffice. There may be instances, however, when carbons buried deep in the core of a molecule will not exhibit any long-range correlations to protons in an HMBC experiment. In such cases, the discrepancy between the number of carbons observed in the 2D spectra and that actually contained in the molecular structure will be highlighted by high-resolution mass spectrometry. Thus, the technology to conveniently acquire a ^{13}C reference spectrum at the submilligram level is quite important; micro dual and heteronuclear nano-probes have been developed by various manufacturers to address this need [6,7]. In the present case, a Nalorac Z•SPEC MD-400 microdual probe was utilized for the acquisition of the ^{13}C reference spectrum shown in Figure 1.

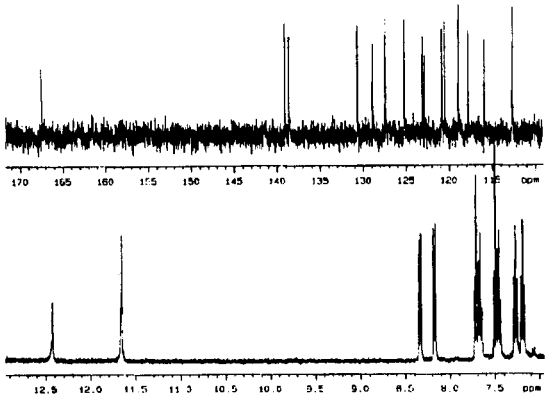


Figure 1. (BOTTOM) Proton reference spectrum recorded for an 800 µg sample of quindolinone (1) in 140 µl of 99.96% d_6-DMSO (Cambridge Isotope Laboratories) at 400 MHz using the decoupler coil of a Nalorac Z•SPEC MD-400-3 microdual probe. (TOP) Carbon reference spectrum recorded for the same sample again using the microdual probe. Acquisition time for the carbon spectrum was 17 hours using a 4 second interpulse delay to facilitate the observation of quaternary carbon resonances. Acceptable quality data could be acquired in less time, if the interpulse delay were shortened and the spectroscopist were willing to accept a lower S/N ratio.

Proton-carbon resonance correlations were established through the acquisition of an HMQC [8] spectrum. The data were acquired as 1024 x (64 x 2) hypercomplex points with 32 transients accumulated per t_1 increment. The data were zero-filled to 2048 x 512 points and Fourier transformed to afford the spectrum shown in Figure 2. Total acquisition time for the spectrum was 32 minutes.

Although no ^{13}C assignments could be made directly from the one-dimensional ^{13}C reference spectrum, the data support an indoloquinoline skeleton when the protonated proton/carbon pair resonating at 7.50/112.8 ppm are considered. The 7.50 ppm proton doublet, which must be one of the terminal spins of one of the two

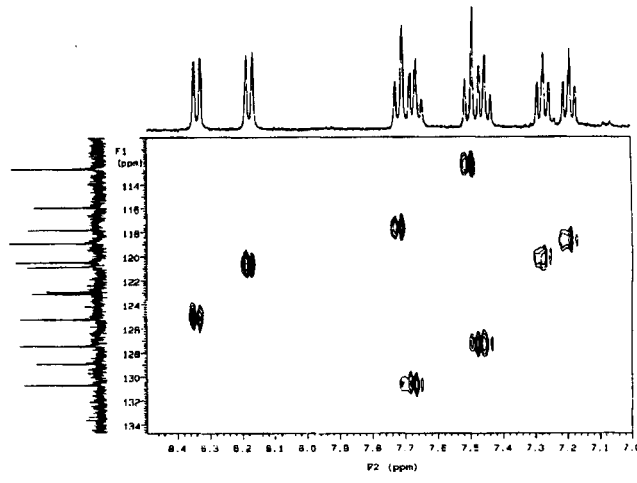


Figure 2. ^1H-^{13}C HMQC spectrum of quindolinone (1) acquired using the pulse sequence of Bax and Subramanian [8] in a Nalorac Z•SPEC MD-400-3 microdual probe. A pair of 1 ms (x,y) trim pulses were applied at a power level 4 dB lower than the "hard" pulses prior to the initiation of the normal pulse sequence. The data were acquired as 2048 x (64 x 2) hypercomplex points [18] with 32 transients/t_1 increment giving an acquisition time of 32 minutes. Data were zero-filled to 2048 x 512 points during processing which employed Gaussian multiplication prior to both Fourier transformations.

four-spin systems, coupled with chemical shift of its directly bound carbon are strongly supportive of what would be the 9-position of the indoloquinoline alkaloids previously isolated form *Cryptolepis sanguinolenta* [10,12]. A second strong piece of evidence in support of this structure is found in the chemical shift of the quaternary carbon resonating at 115.9 ppm. A quaternary aromatic carbon, to exhibit such an upfield chemical shift, must be positioned in the molecular framework such that it is located β to two annular nitrogen atoms. This requirement is met by the quaternary carbon at the C5b position. The corresponding C5b ^{13}C resonances of cryptolepine [10] and quindoline [12] are observed at 113.8 and 121.0 ppm, respectively. No further assignments are possible from the HMQC data.

A pair of HMBC spectra were recorded using long-range delay times of 63 and 83 msec corresponding to 8 and 6 Hz optimization, respectively. The data were essentially comparable and only the 83 msec data are shown in Figure 3. Given the long-range heteronuclear shift correlation information from the HMBC spectra, the two four-spin systems were readily sequenced and complete assignments made. For example, the H1 resonance was unequivocally assigned as the proton resonating at 8.33 ppm through the observation of a $^3J_{CH}$ correlation to the 11-carbonyl carbon resonating at 167.4 ppm. The H1 resonance was also $^3J_{CH}$ correlated to the quaternary carbon resonating at 139.1 ppm, assigned as C4a, and to the protonated carbon resonating at 130.8 ppm, assigned as C3

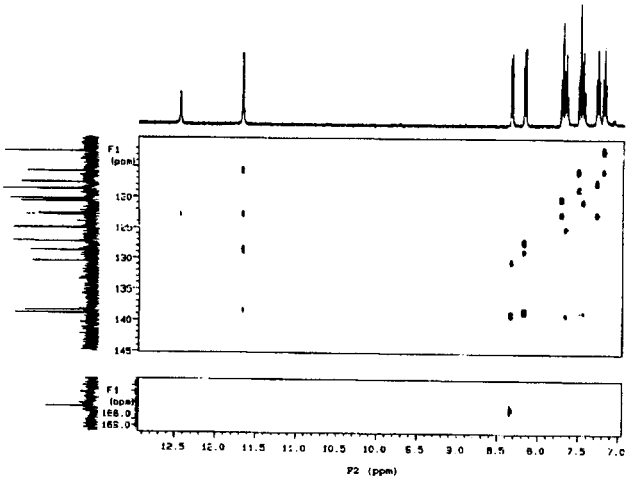


Figure 3. ^{1}H-^{13}C HMBC spectrum of quindolinone (**1**) acquired using the pulse sequence of Bax and Summers [9] in a Nalorac Z•SPEC MD-400-3 microdual probe. The data were acquired as 2048 x (96 x 2) hypercomplex points [18] with 80 transients/t_1 increment, giving an acquisition time of 8.5 hour. Data were zero-filled to 4096 x 512 points during processing which employed Gaussian/shifted Gaussian multiplication prior to the first Fourier transformation and cosine multiplication prior to the second. A normal low-pass J-filter was employed optimized for a 165 Hz $^{1}J_{CH}$ coupling; the long-range delay was set for 83 msec (6 Hz). [A data set was also acquired optimized for 63 msec (8 Hz - not shown)].

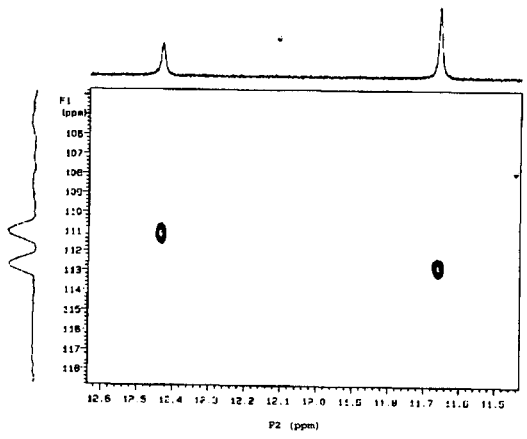


Figure 4. ^{1}H-^{15}N HMQC spectrum of an 800 µg sample of quindoline (**1**) dissolved in 140 µl of d_6-DMSO in a Z•SPEC MID-400 micro inverse-detection probe using the pulse sequence of Bax and Subramanian [8]. The data were acquired as 256 x (30 x 2) hypercomplex points [18] with 64 transients accumulated/t_1 increment. Spectral widths were 836 Hz in F_2 and 1216 Hz in F_1. The experiment was optimized for a $^{1}J_{NH}$ = 96 Hz based on a one-dimensional ^{1}H-^{15}N (^{15}N-coupled) satellite spectrum. A null interval of 0.2 sec was employed with a 1.38 sec interpulse delay. ^{15}N WALTZ decoupling was applied during the 0.153 sec acquisition time. The acquisition time for the phase-sensitive 2D spectrum was 1.7 hours.

and thereby providing the assignment of H3 as the proton resonating at 7.65 ppm. In similar fashion, the balance of the proton and carbon resonance of quindolinone (**1**) were assigned.

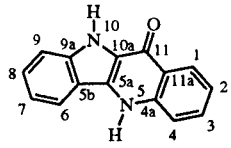
Perhaps the most interesting responses in the HMBC spectrum were those arising from the two NH resonances. The downfield NH, resonating at 12.43 ppm was assigned as the N5-H resonance of the quinolone portion of the molecule through nOe correlations to the H4 and H6 protons resonating at 7.72 and 8.16 ppm, respectively. The indolyl N10 proton resonating at 11.66 ppm exhibited a nOe only to H9 resonating at 7.50 ppm. The quinolone N5-H exhibited a long-range correlation in the HMBC spectrum to the two quaternary carbons resonating near 123 ppm. In contrast, the N10-H indolyl resonance was correlated to the 115.9 ppm C5b quaternary carbon, as well as to one of the quaternary carbons near 123 ppm, and to the 128.9 ppm quaternary carbon, and finally, through a weak correlation to the quaternary carbon resonating at 138.6 ppm. Using other correlations in the spectrum, the N10-H correlates to the four quaternary carbons of the pyrrole ring, C5a, C5b, C9a, and C10a, which resonate at 128.9, 115.9, 138.6 and either 122.9 or 123.1 ppm, respectively. In contrast, the N5-H resonance correlates long-range only to one or both of the quaternary carbons resonating near 123 ppm which are assignable as C10a and C11a. Unfortunately, because of the proximity of the C10a and C11a quaternary carbons, unequivocal assignments cannot be made.

Finally, perhaps the most interesting piece of spectral data to be obtained for **1** was the ^{1}H-^{15}N HMQC spectrum [13] shown in Figure 4. Despite the notorious insensitivity of ^{15}N to direct observation, the nearly 1000-fold enhancement in sensitivity obtained by resorting to indirect detection methods [14,15] makes this experiment a straightforward undertaking even at the 800 µg (3.4 µmoles) level. The ^{1}H-^{15}N HMQC spectrum of **1** was recorded using a Z•SPEC MID-400 micro inverse probe. The data were recorded as 256 x (30 x 2) hypercomplex files. The $^{1}J_{HN}$ heteronuclear shift coupling was optimized for 96 Hz on the basis of a ^{15}N-satellite spectrum. A total of 64 transients were accumulated for each t_1 increments giving a total acquisition time for the phase-sensitive 2D spectrum of 1.7 hours. The N5-quinolone nitrogen resonated at 111.0 ppm; the N10 indolyl nitrogen resonated at 112.6 ppm. These data are reasonably comparable to the $^{15}N10'$ chemical shift of the indolyl NH of cryptospirolepine [11] at 118.9 ppm and those of several other indoloquinolizidine analogs and reserpine, which ranged from 117.9 to 119.6 ppm [16].

Surprisingly, aside from the work cited above, the only other study reporting ^{15}N chemical shift data of alkaloids was found in a paper describing the oxazole-thiazole derived tantazole alkaloids [17]. Unlike the data in this report and our previous work [11], which was performed at natural abundance, the HMQC and HMBC experiments

Table 1

^1H, ^{13}C, and ^{15}N NMR Chemical Shift Data and ^1H-^{13}C Long-Range HMBC Correlations Observed for Quindolinone (1) in d_6-DMSO at 9.4T



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Position	NMR Chemical Shift (δ) ^1H	^{13}C	^{15}N	HMBC Correlations (Protons correlating with a given carbon)
1	8.34	125.2	—	H3
2	7.27	120.5	—	H4
3	7.66	130.7	—	H1
4	7.71	117.8	—	H2
4a	—	139.1	—	H1, H3
5	12.43	—	111.0	—
5a	—	128.9	—	H6 (weak), N10H
5b	—	115.9	—	H7, H9, N10H
6	8.18	120.9	—	H8
7	7.19	118.9	—	H9
8	7.45	127.4	—	H6
9	7.49	112.6	—	H7
9a	—	138.6	—	H6, H8, N10H
10	11.66	—	112.6	—
10a	—	122.9 [a]	—	N5H, N10H
11	—	167.4	—	H1
11a	—	123.1 [a]	—	H2, H4

[a] It is assumed that N5H coupled to both the C10a and C11a carbons, although this could not be confirmed as the two carbons, resonated at 122.9 and 123.1 ppm and thus could not be unambiguously assigned.

described for didehydrotantazole-A were performed on a sample enriched to >90% ^{15}N. While there has thus far been a dearth of ^1H-^{15}N correlation data reported, the superb sensitivity of this experiment by inverse-detection [15], even at relatively low concentrations, can reasonably be expected to lead to more numerous applications of these techniques to alkaloids in the future.

In conclusion, the structure of quindolinone (1) has been elucidated from an 800 μg sample, principally through the use of a Z•SPEC MD-400 microdual probe for both the direct acquisition of a ^{13}C reference and ^1H-^{13}C HMQC and HMBC spectra. A ^1H-^{15}N heteronuclear shift correlation spectrum was also acquired on this sample using a Z•SPEC MID-400 probe. The availability of microprobe technology, in the opinion of the authors, opens the possibility for the elucidation of potentially novel and biologically interesting minor alkaloids which have been heretofore ignored because of the difficulty in isolating adequate samples for conventional structure elucidation methodologies.

REFERENCES AND NOTES

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