Cryptolepinone *vs.* Hydroxycryptolepine: Resolution of a Question of Substituent Functionality and Double Bond Isomerization

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An indolo[3,2-*b*]quinoline alkaloid bearing an *N*-methyl substituent at N5, and an oxygen moiety at the 11-position has been variously described as both cryptolepinone and 11-hydroxycryptolepine by independent research groups. The structure of this alkaloid is unequivocally confirmed as the former, cryptolepinone, with substantial changes in double bond isomerization relative to that which would be required if it were indeed the latter. The structure of the alkaloid was confirmed by, total assignment of the ^{1}H, ^{13}C, and ^{15}N nmr spectra at natural abundance using 3 mm micro inverse nmr probe technology at 400 and 500 MHz.

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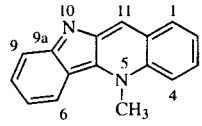
The family of indoloquinoline alkaloids from the indigenous Ghanian shrub *Cryptolepis sanguinolenta* has received increasing attention over the past five years after a relative dearth of earlier reports on these alkaloids. The earliest report of cryptolepine (5-*N*-methylindolo[3,2-*b*]-quinoline, **1**) of which we are aware was the 1951 report of Schlittler and co-workers [1]. Over 25 years elapsed before the next report was to appear in the literature, that of Schiff and colleagues [2], in 1978. Two nmr studies of cryptolepine followed, that of Hufford and co-workers [3] and one from the authors' laboratories [4].

Since these early reports, there have been a burgeoning number of reports dealing with the indoloquinoline and closely alkaloids. In the indolo[3,2-*b*]quinoline family, examples have included quindoline [5], quindolinone [6], and an alkaloid variously described as either cryptolepinone [7,8] or as 11-hydroxycryptolepine [9], which forms the basis for the present study. Examples of the indolo[2,3-*b*]quinoline system have also been reported. Unfortunately, conflicting trivial names for this alkaloid have appeared because of the closely timed submissions: 5-*N*-methylindolo[2,3-*b*]quinoline was named neocryptolepine by Pieters' group [10] in Belgium while the name cryptotackiine was given to this alkaloid by this group [11]. Several synthetic studies on various substituted analogs of the indolo[2,3-*b*]quinoline series have been reported, congeners showing interesting antitumor and DNA topoisomerase II inhibitory activity [12,13]. A very similar situation has also occurred for the indolo-[3,2-*c*]quinoline which has been named crytosanguinolentine [11] by this group and isocryptolepine by Bodo and co-workers [14]. Examples of the indolo[2,3-*c*]quinoline alkaloid skeleton from natural sources remain undiscovered.

In addition to the indoloquinoline alkaloids just briefly reviewed, there have also been examples of closely related systems reported. For example, there have been several reports of indolo[3,2-*b*]benzazepine alkaloids including cryptoheptine [9] and homocryptolepinone [15], which differ in the placement of a hydroxyl/one group. Several dimeric alkaloid systems have also been described. Examples include cryptoquindoline [9], which has since been reported to be an artifact of the isolation scheme [10], biscryptolepine [10], cryptomisrine [16], and cryptolepicarboline [17]. Finally, a unique spiro nona-cyclic alkaloid derived from indoloquinoline and indolobenzazepine components, cryptospirolepine, has also been reported by this group [18].

In terms of reports of long-range ^{1}H-^{15}N heteronuclear shift correlation studies of the indoloquinoline and related alkaloids, there have appeared only three reports in the literature. The first reported only the ^{15}N chemical shift of the protonated indole nitrogen of cryptospirolepine [18]. The chemical shifts of the nitrogens of quindolinone, both protonated, which simplifies their observation considerably, were next reported [6]. Prior to the next reported ^{15}N study of an indoloquinoline alkaloid, gradient-enhanced long-range (GHMBC or GHNMBC) methods were described in the literature. The earliest gradient enhanced long-range ^{1}H-^{15}N experiments were applied to nicotine [19] and ajmaline [20]. Applications to a number of other alkaloids followed in late 1995 continuing through the present [21-27].

Of the long-range ^{1}H-^{15}N studies reported in the literature, a 1996 report detailing the ^{15}N resonance assignments cryptolepine (**1**) [23] is particularly germane to the present study. Interestingly and importantly, the double bond isomerization of cryptolepine, which fixes a double bond between the C9a quaternary carbon and N10 exocyclic to its normal location in the phenyl ring of the indole portion of the molecule, causes a substantial downfield shift of the ^{15}N resonance of N-10 to 207.8 ppm relative to the normal indole/carbazole ^{15}N chemical shift range of approximately 110-140 ppm. In conjunction with the perturbation of the indole N-10 chemical shift, the chemical shift of the C9a quaternary carbon is likewise perturbed by the imine-like exocyclic double bond, shifting downfield to 160.0 ppm relative to the more normal range of 135-140 ppm.



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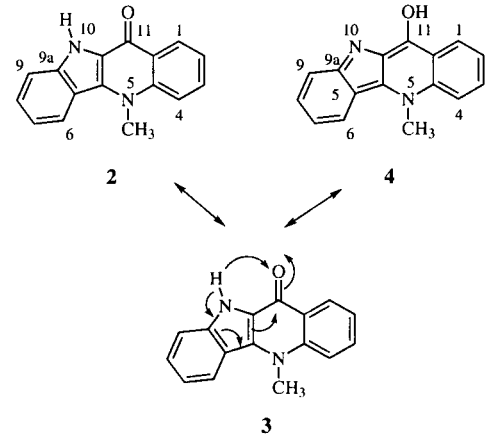
Thus, we now wish to report the results of our study of the ^{1}H, ^{13}C, and ^{15}N chemical shift assigmnents of an alkaloid which we have described as cryptolepinone (**2**) [7], also described as 11-hydroxycryptolepine (**4**) by Paulo, Gomes, and Houghton [9]. We will also address the issue of the correct nature of the oxygen functionality and the double bond isomerization of the molecule. Details of the isolation and the physical constants for the alkaloid have been reported previously [16] and will not be repeated here.

Cryptolepinone (**2**) and 11-hydroxycryptolepine (**4**) can be considered as resonance tautomers of one another. Transfer of the proton from N-10 in **2** to the oxygen functionality at the 11-position accompanied by a series of double bond isomerizations as shown by **3**, affords 11-hydroxycryptolepine (**4**). The question of the nature of the oxygen moiety at the



2 **4**

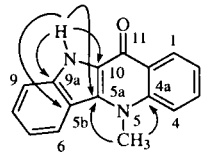
3

11-position of the molecule and the isomerization of the double bonds is readily explained by the total assignment of the ^{1}H, ^{13}C, and ^{15}N resonances of the molecule.

Assignment of the ^{1}H and ^{13}C spectra of the molecule is quite straight-forward. Protons and their directly bond carbons were sequenced using an inverted direct response (IDR)-HMQC-TOCSY spectrum recorded with a mixing time of 24 msec. The eleven protonated aromatic resonances in the molecule were thus grouped into two four-spin systems. Although the IDR-HMQC-TOCSY data correctly sequences the contiguous protonated carbons, these data cannot correctly orient the four-spin systems relative to the carbon skeleton of the molecule.

Orientation of the two four-spin systems to the carbon skeleton was readily accomplished from a single nOe difference spectrum in which the *N*-methyl resonance was irradiated. NOe's were thus observed to the peri H4 and H6 resonances which flank the *N*-methyl group. From this experiment, the protons exhibiting nOe's, which resonated at 7.96 and 8.38 ppm gave nOe's of 7.4 and 7.1%, respectively. The 7.96 and 8.38 ppm resonances were directly bound to carbons resonating at 116.0 and 123.0 ppm, respectively. A second nOe experiment irradiated the proton resonance furthest downfield which we attribute to the N-10-H resonance at 11.95 ppm. In this experiment, a 12.1% nOe was observed to the proton resonating at 7.58 ppm. In concert, the results of these two irradiations establish the proton at 7.58 ppm as H9, assuming the proton resonating at 11.95 ppm to indeed be on N-10. Tracking correlations through the contiguous protonated carbon resonances with the IDR-HMQC-TOCSY experiment, the proton resonating at 8.38 ppm is assigned as H6. Finally, the proton resonating at 7.96 ppm is assigned as H4. Of course all of these assignments are contingent upon the proton at 11.95 ppm residing on N-10. If the converse, as proposed by Paulo, Gomes, and Houghton [9] were true, *i.e.* the downfield proton residing on the oxygen functionality at the 11-position as an OH, then the proton at 7.58 ppm would necessarily be assigned as H1, the resonance at 8.38 ppm as H4, and the resonance at 7.96 ppm as H6.

In part, the question of the location of the proton resonating at 11.95 ppm residing on nitrogen *vs.* oxygen is resolved from the long-range ^{1}H-^{13}C HMBC correlations. First, the *N*-methyl resonating at 4.35 ppm, is long-range coupled to a pair of quaternary carbons resonating at 141.0 and 131.0 ppm, which must necessarily be C4a and C5a. The former is assigned as C4a and the latter as C5a. This assignment is based on the well established upfield shift of carbons β to nitrogen substituents which generally ranges from about 10-15 ppm. Next, the downfield proton resonance at 11.95 ppm is correlated to four quaternary carbons that resonate at 131.0, 116.5, 139.0, and 124.0 ppm. The first of these carbon resonances can reasonably couple *via* $^{3}J_{CH}$ to the proton resonating at 11.95 ppm if this proton is indeed on N-10. This correlation is considerably less reasonable in a non-gradient HMBC experiment if the proton in question is on oxygen, in which case the correlation would be *via* $^{4}J_{CH}$. The carbon resonating at 116.5 ppm correlates to two protons which resonate at 7.58 and 7.19 ppm. The former we have tentatively assigned as H9. The latter resonance, the furthest upfield shifted aromatic resonance, is assigned as H7, the chemical shift is fully consistent with what would be the 5-position of a simple indole. The carbon resonating at 139.0 ppm is tentatively assigned as C9a and couples to the H6 proton resonating at 8.38 ppm and to a proton resonating at 7.48 ppm which is assigned as H8 in addition to the coupling to the resonance we attribute to N-10H. The latter coupling, of course, would be *via* $^{2}J_{CH}$ which is not uncommon in five-membered heterocycles such as pyrrole, furan, and thiophene and their respective benzo analogues. Finally, the carbon resonating at 124.0 ppm is coupled only to the proton resonating at 11.95 ppm, which would be consistent if this resonance is assigned as C10a since the only coupling pathway available to that carbon would be to the N-10H resonance again *via* $^{2}J_{CH}$. Correlations to the quaternary carbons for the proposed structure of cryptolepinone (**2**) are shown by **5**.



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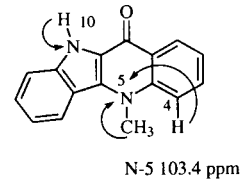
The balance of the long-range heteronuclear correlations in the 400 MHz HMBC spectrum were fully consistent with the proposed structure of **2**. Perhaps the most compelling resonance assignment is that of the C9a resonance at 139.0 ppm, which is well within the normal range for the nitrogen-bearing carbon at this position of an indole system. Paulo, Gomes, and Houghton [9], who acquired data for this compound in a deuteriochloroform:perdeuteriomethanol (2:1) solvent mixture also report the chemical shift of this position as 139.21 ppm. The reported chemical shift of C9a for the structure of cryptolepinone (**2**) would be fully consistent with expectation. In contrast, if the correct structure were that of 11-hydroxycryptolepine (**4**), then the chemical shift of C9a would be expected to be more imine-like, *i.e.* more similar to the chemical shift of C9a in cryptolepine which we have reported [4] at 159.95 ppm in dimethyl-d_6 sulfoxide, which Hufford and coworkers [3] assign at 161.0 ppm in deuteriochloroform, and which Paulo, Gomes, and Houghton [9] assign at 161.46 ppm again in deuteriochlorofom than to a simple indole C9a resonance.

Irrefutable resolution of the question of the nature of the oxygen functionality at the 11-position and the consequent fixed isomerization of the double bond system of the molecule that would be required to locate a hydroxyl group at the 11-position is provided by ^{15}N nmr data. First, a 500 MHz one-dimensional ^{1}H-^{15}N satellite spectrum was acquired, the one-bond coupling delay optimized for 90 Hz. The downfield singlet gave a doublet in the ^{15}N satellite spectrum with a coupling constant $^{1}J_{NH}$ = 97.9 Hz. This data alone irrefutably locates the proton in question on N-10. In addition, however, a long-range ^{1}H-^{15}N GHMBC spectrum was also acquired, the long-range delay optimized for 3 Hz. Responses to two nitrogen resonances were observed. An ~98 Hz doublet was observed in the spectrum at the proton shift of the N-10H resonance and was associated with a nitrogen resonating at 113.1 ppm (downfield from liquid NH_3). The observed chemical shift is consistent with the general 110-140 ppm range of ^{15}N chemical shifts for indole and carbazole analogues (^{15}N indole 130.5 ppm [28, 29]; ^{15}N carbazole 111.5 ppm [29]). No other correlations were observed to this nitrogen resonance. The other nitrogen, N-5 which resonated at 103.4 ppm, exhibited correlations to the *N*-methyl singlet resonating at 4.35 ppm and to the H4 proton resonating at 7.96 ppm.

N-10 113.1 ppm



N-5 103.4 ppm

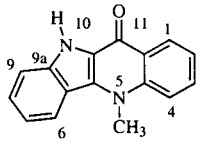
Comparing the ^{15}N resonance assignments of **2** to those of cryptolepine (**1**) [23], the lack of congruence between the C9a carbon chemical shift of **2** and that of cryptolepine (**1**) carries through into the nitrogen spectra as well. While the protonated N-10H ^{15}N resonance was observed at 113.1 ppm, the corresponding N-10 resonance of cryptolepine was observed at 207.8 ppm, which is consistent with its imine-

like character. As shown previously [23], the observed chemical shift of N-10 of **1** was consistent with comparable nitrogens with fixed exocyclic C=N structures [30].

In conclusion, we have irrefutably shown the structure of an alkaloid isolated from *C. sanguinolenta* that has been variously described as cryptolepinone [7,8] and as 11-hydroxycryptolepine [9] to be correctly assigned as the former through the total assignment of the ^{1}H, ^{13}C, ^{15}N spectra of the molecule which are collected in Table 1, accompanied by the ^{1}H and ^{13}C resonance assignments reported for this alkaloid in a 2:1 deuteriochloroform:perdeuteriomethanol solvent mixture. The fixed exocyclic isomerization of the double bond system of cryptolepine (**1**), which locates a C=N bond between C9a-N10, has been shown to be a diagnostically useful characteristic of cryptolepine, obviating the necessity of exploring the ^{15}N spectra of related alkaloids under normal circumstances.

Table 1

^{1}H, ^{13}C , and ^{15}N Resonance Assignments for Cryptolepinone (**2**) and Corresponding Assignments Proposed by Paulo, Gomes, and Houghton [9] for 11-Hydroxycryptolepine (**4**)



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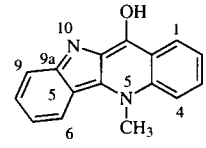

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	Present Study (Dimethyl-d_6 Sulfoxide)			Paulo, Gomes, and Houghton [9] Deuteriochloroform: perdeuteriomethanol (2:1)	
position	$\delta^{1}H$ [a]	$\delta^{13}C$	$\delta^{15}N$ [b]	$\delta^{1}H$	$\delta^{13}C$
1	8.43	126.0		8.47	125.03
2	7.36	121.0		7.31	121.32
3	7.78	131.9		7.69	130.49
4	7.96	116.0		7.45	114.04
4a	--	141.0	--	--	139.51
N-5	--	--	103.4	--	-
N-Me	4.35	36.3	--	4.39	36.38
5a	--	131.0		--	131.47
5b	--	116.5		--	115.53
6	8.38	123.0		8.19	122.14
7	7.19	119.8		7.65	119.22
8	7.48	127.0		7.75	127.36
9	7.58	113.0		7.72	112.01
9a	--	139.0	--	--	139.21
N-10H	11.95	--	113.1	10.17 [c]	--
10a	--	124.0	--	--	123.19
11		167.4			167.01
11a		123.8			122.87

[a] ^{1}H and ^{13}C chemical shift assignments were established from a series of nOe difference spectra, an IDR-HMQC-TOCSY spectrum with a 24 msec mixing time, and a 10 Hz HMBC spectrum. The data were acquired for a sample consisting of 10 mg of **2** dissolved in 160 µl dimethyl-d_6 sulfoxide. Experiments were performed on a Varian Unity 400 MHz spectrometer operating at 399.80 MHz for ^{1}H observation and equipped with a Nalorac Z•SPEC™ MID400-3 micro inverse probe. [b] ^{15}N chemical shifts were measured using the same sample as above. The ^{15}N satellite spectrum was optimized for an assumed 90 Hz $^{1}J_{NH}$ coupling. The long-range ^{1}H-^{15}N GHMBC experiment was optimized for 3 Hz. The nitrogen experiments were performed on a Varian Unity 500 NMR spectrometer operating at 499.75 MHz for ^{1}H observation and equipped with a Nalorac Z•SPEC™ MIDTG-500-3 gradient micro inverse probe. The satellite spectrum was acquired in ~30 minutes; the GHMBC spectrum was acquired overnight as 8192 x 32 hypercomplex files. The GHMBC data were processed using sinebell multiplication prior to the first Fourier transform. The data were linear predicted to 128 files in F_1 and were cosine multiplication prior to the second transform. [c] The resonance assignment shown is as the N-10H resonance as has been shown to be correct. The resonance was originally assigned as the 11-OH resonance [9].

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