Two New Indoloquinoline Alkaloids from *Cryptolepis sanguinolenta*: Cryptosanguinolentine and Cryptotackieine

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Received August 15, 1995

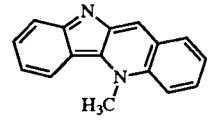
The roots of the indigenous West African shrub *Cryptolepis sanguinolenta* have proved to be a rich source of indoloquinoline alkaloids. To date, all of the alkaloids isolated have been analogs of indolo[3,2-*b*]quinoline. We now wish to report examples of two new indoloquinoline alkaloids which differ in the fusion of the indole and quinoline rings. The first, cryptosanguinolentine, is an angular indolo[3,2-*c*]quinoline. The second, cryptotackieine, is a linear indolo[2,3-*b*]quinoline system. Both of these families of alkaloids are without precedent from *C. sanguinolenta*. The structures of both were established through the extensive use of inverse-detected micro nmr methods.

J. Heterocyclic Chem., **33**, 239 (1996).

Introduction.

The West African plant *Cryptolepis sanguinolenta* has been traditionally used by Ghanaian healers to treat a variety of disorders [1]. Previous studies of the alkaloidal content of this plant led to the isolation and identification of cryptolepine (**1**) [2-5] and quindoline (**2**) [3,6]. More recently, the complex spiro nonacyclic alkaloid cryptospirolepine (**3**) was isolated and its structure determined through the extensive use of two-dimensional nmr spectroscopy and mass spectrometery [7]. Submilligram amounts of quindolinone (**4**) were isolated and the structure determined [8]. Finally, a submicromole (~0.25 μmole) quantity of the dimeric alkaloid cryptolepicarboline (**5**) was isolated and characterized [9]. Despite the small quantity of material isolated, it was sufficient for the determination of the structure of cryptolepicarboline (**5**) through the extensive use of inverse-detected micro nmr techniques. The structure of **5** incorporated a β-carboline linked *via* N9' to the 11-position of a cryptolepine-like indoloquinoline nucleus.

It is interesting to note that all of the alkaloids isolated from *C. sanguinolenta* thus far were based-on or derived-from an indolo[3,2-*b*]quinoline skeleton. We now report the isolation and structure elucidation of two additional minor alkaloids, cryptosanguinolentine (**6**), an angular isomeric indolo[3,2-*c*]quinoline and cryptotackieine (**7**), a linear indolo[2,3-*b*]quinoline. To our knowledge, both of these alkaloids contain ring systems that have heretofore



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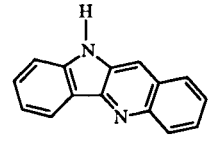

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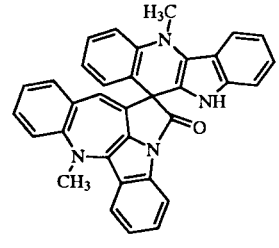

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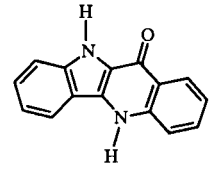

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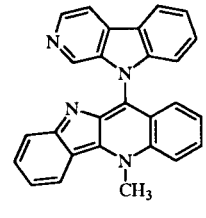

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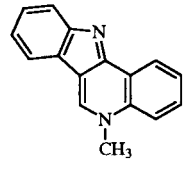

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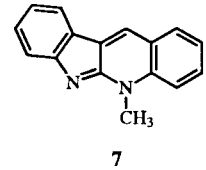

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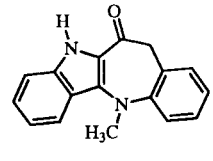

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not been encountered either in this genus or in other higher plants. It is also interesting to speculate that the isolation of cryptosanguinolentine (**6**) and cryptotackieine (**7**) may perhaps herald the presence of other equally interesting alkaloids in *C. sanguinolenta*.

In particular, it remains to be explained how the benzpyrrolizino-benzazepine (lower subunit) of cryptospirolepine (**3**) is biosynthetically elaborated, suggesting that the plant may also produce indolobenzazepine alkaloids. One benzazepinone alkaloid, homocryptolepinone (**8**), which could reasonably serve as a biosynthetic precursor of the benzpyrrolizinobenzazepine portion of cryptospirolepine, has recently been characterized [10], at least partially confirming our suppositions regarding the biogenesis of the benzpyrrolizinobenzazepine portion of cryptospirolepine.

Results and Discussion.

One- and two-dimensional nmr spectral data were used in concert to establish the structures of cryptosanguinolentine (**6**) and cryptotackieine (**7**). Applications of inverse-detected 2D nmr in natural products chemistry [11,12] and more specifically alkaloid structure determination specifically have recently appeared [13]. Augmented by the increased sensitivity afforded by micro-probes [14,15], the acquisition of sufficient spectral data to determine the structure of very minor natural products can be conducted in a time-efficient manner. Given the 1.7 and 0.5 mg samples of **6** and **7**, respectively, that were available, the only time-consuming step in the nmr data acquisition was the acquisition of ^{13}C reference spectra. Complete details of the elucidation of the structures of **6** and **7** are presented below.

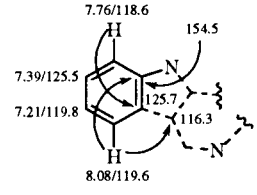
Cryptosanguinolentine.

Cursory inspection of the 400 MHz proton spectrum of **6** showed it to be composed of two four-spin systems, an isolated aromatic singlet, and a singlet which could arise from either an *O*- or *N*-methyl resonance at 4.26 ppm. The proton spins were readily sequenced using a COSY spectrum. Through-space correlations were established using a NOESY spectrum. The ^{13}C spectrum of the molecule confirmed the methyl group as an *N*-methyl based on the methyl carbon chemical shift of 42.2 ppm. The aromatic region of the ^{13}C spectrum contained a total of sixteen resonances; nine were protonated carbons and six were quaternary carbons. The one-dimensional reference spectra are plotted flanking the inverse-detected heteronuclear spectra discussed below (see Figures 1 and 2).

One-bond proton-carbon shift correlations were established from the HMQC [16] spectrum. Long-range heteronuclear correlations were obtained from HMBC [17] spectra recorded with 63 (8 Hz) and 83 (6 Hz) msec optimizations.

Three-bond ($^3J_{CH}$) long-range heteronuclear correlations, which dominated the HMBC spectrum, located the quaternary carbons relative to the two four-spin systems. The proton-carbon resonant pair at 7.76/118.5 ppm suggested a chemical environment analogous to that of the 10-position of cryptolepine [5], and provided a starting point for the structure elucidation. Operating on the premise that the molecule was indoloquinoline-derived, a quaternary carbon long-range coupled to the proton which resonated at 7.76 ppm would necessarily be located beta to the indole nitrogen.

The four-spin system containing the proton doublet resonating at 7.76 ppm was sequenced from a COSY spectrum and confirmed by long-range correlations contained in the HMBC spectrum, as shown by **9**. The proton which resonated at 7.76 ppm was long-range coupled to a quaternary carbon that resonated at 125.7 ppm, consistent with a location in the carbon skeleton of the molecule beta to nitrogen. Two quaternary carbons were long-range coupled to the proton doublet that resonated at 8.08 ppm at the other terminus of the four-spin system. One quaternary carbon resonated at 116.3 ppm, suggesting a location in the skeletal structure beta to two nitrogen atoms. This environment would be analogous to that of the C5b position of cryptolepine [5]. The second quaternary carbon resonated at 154.5 ppm which was consistent with a carbon bound directly to nitrogen. The chemical shift, however, was atypical of those of either cryptolepine [5] or quindoline [6], which resonated at 160.0 and 144.1 ppm, respectively.



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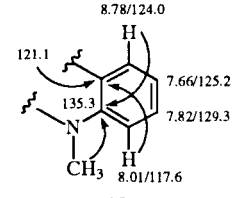

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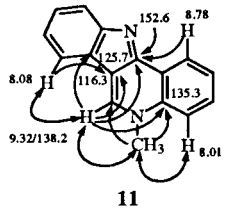
Correlations from the proton which resonated at 8.78 ppm and the *N*-methyl group assigned the 135.3 ppm quaternary carbon resonance as C4a. The H4 proton, which resonated at 8.01 ppm, was long-range coupled to the C11a quaternary carbon that resonated at 121.1 ppm, as shown by **10**.

Remaining to be assigned were two quaternary carbon resonances and the protonated carbon resonance associated with the proton singlet (9.32/138.2 ppm). The latter, based on the chemical shift of both the proton and carbon, was suggestive of a nitrogen-bearing protonated carbon. Long-range correlations from the 9.32 ppm singlet were observed to the quaternary carbons resonating at 116.3, 125.7, 152.6, and 135.3 ppm. The only long-range corre-

lation to the carbon resonating at 138.2 ppm was from the *N*-methyl resonance. It was also noted from the HMBC data that the proton resonating at 8.78 ppm (H1) is also long-range coupled to the quaternary carbon resonating at 152.6 ppm. These data, when interpreted in unison, allowed the postulation of a 5-methyl-5*H*-indolo[3,2-*c*]-quinoline carbon skeleton represented by **11.**



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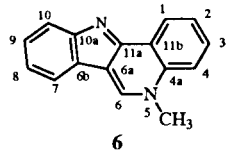
Connectivity information contained in the NOESY spectrum was used to further support the proposed indolo[3,2-*c*]quinoline skeleton. Correlations in the NOESY spectrum (double-headed arrows) were observed between the singlet resonating at 9.32 ppm, the proton resonating at 8.08 ppm, and the *N*-methyl resonating at 4.26 ppm. The *N*-methyl was, in turn, correlated to the proton resonating at 8.01 ppm in the other four-spin system. The nOe correlations extracted from the NOESY spectrum are shown by **11**. Complete resonance assignments and long-range heteronuclear shift correlations are presented in Table 1.

Table 1

Proton and Carbon Chemical Shift Assignments and Long-range Connectivities Observed in the HMBC Spectrum of Cryptosanguinolentine (**6**)



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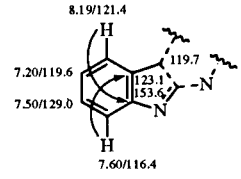
Position	NMR Chemical Shift (ppm) ^1H	^{13}C	Long-range Couplings [a]
1	8.78	124.0	H3
2	7.66	125.2	H4
3	7.82	129.3	H1
4	8.01	117.6	H2
4a	—	135.3	H1, H3, H6, NCH_3
6	9.32	138.2	NCH_3
6a	—	116.3	H7, H6 ($^2J_{CH}$)
6b	—	125.7	H6, H10, H10
7	8.08	119.6	H9
8	7.21	119.8	H10
9	7.39	125.5	H7
10	7.76	118.6	H8
10a	—	154.5	H7, H9
11a	—	152.6	H1, H6
11b	—	121.1	H2, H4
NCH_3	4.22	42.3	—

[a] Long-range couplings are specified from the proton indicated to the carbon at a given position.

Cryptotackieine.

Except for differences in chemical shifts, the constituent resonances in both the proton and carbon spectra of cryptotackieine (**7**) were qualitatively similar to those of cryptosanguinolentine (**6**). The HMQC [16] and HMBC [17] spectra were used to establish one-bond and long-range heteronuclear correlations, respectively. The proton resonances of the two four-spin systems were sequenced using a COSY spectrum and confirmed by long-range correlations in the HMBC spectrum. In a manner analogous to that described above, quaternary carbon resonances were located in the carbon skeleton.



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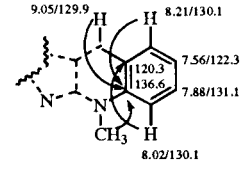

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It was interesting to note that the quaternary carbon chemical shifts of cryptotackieine (**7**) closely paralleled those of cryptosanguinolentine (**6**). The proton resonating at 7.60 ppm was long-range coupled to a quaternary carbon which resonated at 123.1 ppm, located beta to a nitrogen based on its chemical shift. The proton at the other terminus of that four-spin system, which resonated at 8.19 ppm, was long-range coupled to a quaternary carbon resonating at 153.6 ppm. The chemical shift of the latter, again suggested an imine-like structure for the nitrogen as shown by **12**. In similar fashion, the quaternary carbon resonating at 136.6 ppm was long-range coupled to the *N*-methyl, as well as to the proton doublet resonating at 8.21 ppm and to the proton singlet resonating at 9.05 ppm, as shown by **13**. Of the two remaining quaternary carbons, which resonated at 154.5 and 119.7 ppm, the former was long-range coupled to both the *N*-methyl and the proton singlet resonating at 9.05 ppm. The latter exhibited no long-range couplings. Interpretation of all of the long-range coupling information in conjunction with through-space correlations

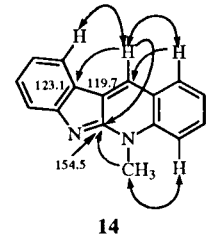

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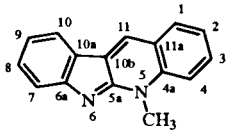
Table 2

Proton and Carbon Chemical Shift Assignments and Long-range Connectivities Observed in the HMBC Spectrum of Cryptotackieine (7)



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Position	NMR Chemical Shift (ppm) ^1H	^{13}C	Long-range Couplings [a]
1	8.21	130.1	H3, H11
2	7.56	122.3	H4
3	7.88	131.1	H1
4	8.02	115.0	H2
4a	—	136.6	H1, H3, H11, NCH_3
5a	—	154.5	H11, NCH_3
6a	—	153.6	H8, H10
7	7.60	116.4	H9
8	7.50	129.0	H10
9	7.20	119.6	H7
10	8.19	121.4	H8
10a	—	123.1	H7, H9, H11
10b	—	119.7	—
11	9.05	129.9	H1
11a	—	120.3	H2, H4
NCH_3	4.32	33.2	—

[a] Long-range couplings are specified from the proton indicated to the carbon at a given position.

obtained from one-dimensional nOe difference spectra allowed the assembly of the structure of cryptotackieine (7), an indolo[2,3-*b*]quinoline, which had no prior precedent from *C. sanguinolenta*.

Complete proton and carbon resonance assignments and the long-range couplings observed in the HMBC spectrum are presented in Table 2.

Conclusions.

Two new alkaloids were isolated from extracts of the roots of the indigenous West African shrub *Cryptolepis sanguinolenta* and their structures determined through the use of inverse-detected micro nmr techniques. The first new alkaloid, cryptosanguinolentine (**6**), was an angular indolo[3,2-*c*]quinoline. The second new alkaloid, cryptotackieine (**7**), was a linear indolo[2,3-*b*]quinoline system related to cryptolepine (**1**) an indolo[3,2-*b*]quinoline. The indolo[2,3-*b*]-ring system differs from the indolo[3,2-*b*]-quinoline in the fusion of the indole to the quinoline nucleus. While the two annular nitrogens of cryptolepine, quindoline and related *Cryptolepis* alkaloids are on opposite sides of the tetracyclic system, both annular nitrogens of cryptotackieine are on the same side of the linear tetracyclic skeleton. Both of the indoloquinoline alkaloids described in this work represent ring systems which were without precedent from extracts of *C. sanguinolenta*.

EXPERIMENTAL

Isolation.

Cryptosanguinolentine (**6**) and cryptotackieine (**7**) were isolated as yellow and yellowish-green residues consisting of 1.7 and 0.5 mg, respectively, by repeated column chromatography of the alkaloid fraction of an ethanol extract of the roots (3.14 kg) of *C. sanguinolenta* over neutral alumina, followed by repeated preparative reversed-phase hplc.

NMR Spectroscopy.

All nmr spectra were obtained by dissolving samples of **6** and **7** in 140 µl of DMSO-d_6 which was then transferred to a 3 mm nmr tube using a flexible Teflon needle (Wilmad) and a Hamilton gas-tight Leur-lok syringe. All nmr data were acquired using a Varian Unity 400 spectrometer operating at a proton observation frequency of 399.952 MHz and equipped with an Z•SPEC® MD-400-3 microdual or MID-400-3 micro inverse probes obtained from Nalorac Cryogenics Corp., Martinez, CA.

The high-resolution proton spectra of **6** and **7** were both recorded in 8 transients and are shown plotted above their respective HMQC and HMBC spectra. NOe difference spectra recorded for both compounds were recorded using a 6 second irradiation of the proton of interest. Data were acquired using 8192 points, which gave an acquisition time of approximately 1 second. Difference spectra were calculated by subtracting a reference free-induction-decay (fid) obtained by irradiation 2 KHz downfield of the lowest field alkaloid resonance, from the fid obtained by on-resonance irradiation. The ^{13}C reference spectra were recorded at an observation frequency of 100.577 MHz using a 35° 4 µsec pulse and a 1.5 second interpulse delay. The spectra of both alkaloids were digitized using 61888 data points across a spectral window of 20629 Hz; the data were zero-filled to 128K points prior to Fourier transformation. Spectra were acquired in 5696 and 26112 transients for **6** and **7**, respectively. The ^{13}C reference spectrum are plotted vertically flanking their respective HMQC and HMBC spectra.

COSY spectra of the aromatic regions of both alkaloids were acquired as 512 x 160 data points using 16 and 32 transients/t_1 increment. The data were zero-filled to 1024 x 1024 points during processing and were symmetrized prior to plotting. A NOESY spectrum was acquired for **6** and utilized a spectral window of 2580 Hz to include the *N*-methyl resonance. The data were taken as 1472 x (64 x 2) hypercomplex points. A total of 64 transients were accumulated for each increment of t_1. The mixing time was 400 mseconds with an interpulse delay of 2.5 seconds. The phase-sensitive data were zero-filled to 2K x 2K points prior to Fourier transformation and were not symmetrized.

The phase-sensitive HMQC spectra of **6** and **7** were acquired using the pulse sequence of Bax and Subramanian [16] modified according to the method of Marion, *et al.* [18] to shift residual axial peaks to the edges of the spectrum. Data for both compounds were acquired 1024 x (100 x 2) hypercomplex files with 16 and 32 transients/t_1 increment for **6** and **7**, respectively. F_2 spectral widths were 2580 Hz; the F_1 spectral widths were 10359 Hz. An interpulse delay of 2.2 seconds was employed. Data were subjected to gaussian multiplication prior to both Fourier transforms and were zero-filled to 2048 x 256 points.

The HMBC spectra were acquired as 2048 x (128 x 2) hypercomplex points using the pulse sequence of Bax and Summers

[17], again modified as in the work of Marion, *et al.* [18] to shift any residual axial peaks to the edges of the spectrum. Spectral widths were 2580 and 4425 Hz in F_2 and F_1, respectively, for both compounds. The low-pass J-filter was optimized for an assumed $^1J_{CH}$ = 165 Hz; the long-range delay was optimized for either 63 (8 Hz) or 83 (6 Hz). A total of 192 transients were accumulated/t_1 increment. The data were subjected to Gaussian multiplication in t_2 and cosine multiplication in t_1, zero-filled to 4096 x 512 points and then mixed-mode processed (absolute value in F_2 and phase-sensitive in F_2) [19].

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