Isolation and Elucidation of the Structure of Homocryptolepinone

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The structure of a new indolobenzazepine alkaloid, homocryptolepinone, isolated from extracts of the roots of the indigenous Ghanaian medicinal plant *Cryptolepis sanguinolenta*, is reported. The structure was determined using mass spectrometric, one-dimensional nOe difference nmr, and inverse-detected two-dimensional nmr experiments which included HMQC, IDR-(Inverted Direct Response)-HMQC-TOCSY, and HMBC experiments. The structure of homocryptolepinone is significant in that it may provide insight into the biogenesis of the benzpyrrolizinobenzazepine portion of the structure of the complex spiro nonacyclic alkaloid cryptospirolepine previously isolated in these laboratories from *C. sanguinolenta,* and which has no precedent in alkaloid chemistry.

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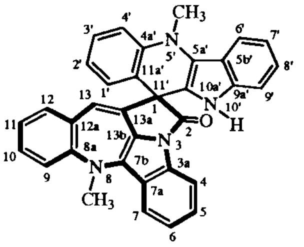
Introduction.

Several major alkaloids, cryptolepine and quindoline, have been repeatedly isolated from extracts of the indigenous Ghanaian medicinal plant *Cryptolepis sanguinolenta,* and rigorously characterized [1-4]. More recently, two other complex alkaloids have also been isolated from the same plant. These included the unique spiro-nonacyclic alkaloid cryptospirolepine (**1**) [5] and the dimeric alkaloid cryptolepicarboline (**2**) [6]. Both **1** and **2** are derived from an indoloquinoline nucleus common to cryptolepine and quindoline, however both are unique in their own right. Cryptospirolepine (**1**) incorporated a benzpyrrolizinobenzazepine (denoted by darkened bonds, **3**) moiety that is spiro-fused to the 11-position of an indoloquinoline nucleus. Cryptolepicarboline (**2**) was comprised of a β-carboline linked to the 11-position of the indoloquinoline nucleus *via* a single bond from the N9' position of the β-carboline. To the best of our knowledge, the benzpyrrolizinobenzazepine (**3**) subunit of **1** is without precedent. Biogenetically, it would also be difficult to rationalize the derivation of **3** from a simple indoloquinoline precursor. However, the isolation of homocryptolepinone (**4**) from *C. sanguinolenta,* which we now report, may ultimately facilitate an explanation of the biogenesis of **3** and hence cryptospirolepine (**1**) through reactions involving the 11-carbonyl and the indole-NH of **4**.



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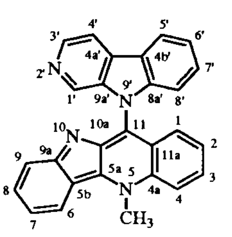
1 (full structure)
3 (bonds shown in bold only)



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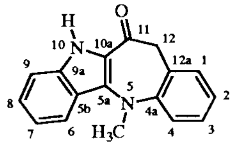

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Isolation.

Column chromatography of the basic fraction of a defatted ethanolic extract of the roots of *C. sanguinolenta* (Lindl.) Schlechter (Asclepiadaceae) over alumina followed by elution with petrol-chloroform (1:2) afforded homocryptolepinone (**4**) as a crude orange residue.

Following thin-layer chromatography over silica gel, the alkaloid stained reddish-brown on exposure to Ehrlich reagent, while failing to stain with Dragendorff reagent [7]. The alkaloid was purified by repeated column chromatography over silica gel followed by preparative hplc, to afford **4** as fine greenish-yellow needles (3.5 mg) that crystallized from methanol. The uv spectrum (chloroform) of the alkaloid was characterized by absorption maxima at 246 nm (log ε 3.33), 269 (3.35), 307 (sh, 3.13), 319 (3.17), and 390 (2.99), with no bathochromic shift upon the addition of a small amount of 0.1*N* methanolic hydrochloric acid. The FT-IR spectrum displayed significant absorption bands at 1682, 1614, 1556, 1520, 1484, 1344, 763, and 738 cm^{-1}.

Structure Elucidation.

The structure of homocryptolepinone (**4**) was deduced through apci-ms (atmospheric pressure chemical ionization-mass spectrometry) and extensive use of inverse-detected two-dimensional nmr experiments. Ions $(M+1)^+$ and $(M-1)^-$ were observed in the positive and negative ion apci-ms at 263 and 261 Da, respectively. The $(M+1)^+$ ion was the base peak in the positive ion spectrum with relatively little fragmentation. The sole major fragment ion was observed at m/z 117 (35.3%), with other fragment ions being found at m/z 107 (4.7), 219 (3.4), 233 (3.9), and 248 (2.1). In contrast, in the negative ion apci-ms, the base peak was m/z 246 while the $(M-1)^-$ peak relative intensity was 32%. Other fragment ions, all again <10% relative intensity, were observed at m/z 148 (2.1), 212 (3.0), 217 (9.1), 231 (4.4). These data were characteristic of an indoloquinoline-derived molecule +30 mass units, presumably accounted for by a carbon, an oxygen, and two hydrogens.

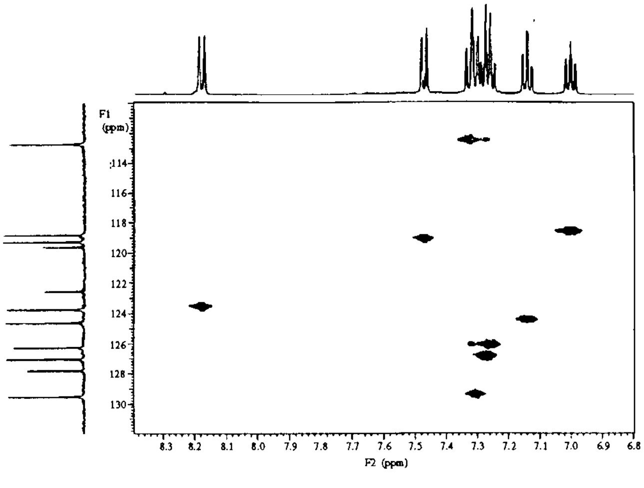


Figure 1. HMQC [8] spectrum of homocryptolepinone (**4**) recorded in d_6-DMSO at 500 MHz. The spectrum is flanked by a 500 MHz high resolution proton reference spectrum plotted horizontally above the contour plot and by a 75 MHz ^{13}C reference spectrum plotted vertically.

The 500 MHz proton spectrum of **4** (shown in Figure 1 plotted above the HMQC spectrum) contained resonances consistent with an NH (11.21 ppm), eight aromatic protons (8.18, 7.47, 7.33, 7.30, 7.27, 7.26, 7.14, and 7.00 ppm), a three proton singlet at 3.97 ppm, which was reasonable for an $N-CH_3$ in the indoloquinoline series, and finally a two proton singlet at 3.62 ppm. The chemical shift of the latter was suggestive of a methylene located either between two aryl systems or between an aromatic ring and a carbonyl. When the proton resonance data were considered in conjunction with the apci-ms data, both could be accounted for by an indoloquinoline nucleus plus a carbonyl and two protons. One of the protons, obviously, may be accounted for by the NH, relative to cryptolepine which lacks an NH [3], and this was reasonable since homocryptolepinone did not exhibit the deep violet color consistent with the extended conjugation conferred by the exocyclic imine C=N double bond. The second hydrogen can presumably be accounted for in the methylene resonance. It was most convenient, as an initial starting premise, to locate this hydrogen atom at the 11-position of a cryptolepine nucleus para to the *N*-methyl substituent, since there was no aromatic proton singlet, which would normally be required to account for an aromatic proton at the 11-position. These arguments provided a starting point for the structure elucidation, requiring the deduction of the location of the carbonyl moiety somewhere in the carbon framework of **4**. It was also important to note that **4** exhibited signs of degradation in d_6-DMSO, suggesting that was prone to either air- or solvent-induced oxidation.

Direct (one-bond) proton-carbon chemical shift concordance was established from an HMQC [8] spectrum shown in Figure 1. To preserve F_1 digital resolution in the congested aromatic region, the F_1 spectral window was restricted from 100-140 ppm, intentionally folding the two aliphatic resonances. Aliphatic carbon resonance chemical shifts were initially obtained by frequency shifting the spectrum in the F_1 domain and were subsequently confirmed from a ^{13}C reference spectrum acquired at 75 MHz using a 3 mm tube in a 5 mm quad-nucleus probe (also shown in Figure 1 plotted vertically flanking the HMQC spectrum).

Assembly of the structure of homocryptolepinone (**4**) was accomplished by first sequencing the protonated carbon resonances with an IDR-HMQC-TOCSY [9] spectrum as shown in Figure 2. The two heteronuclear spin systems, each containing four contiguous protonated carbons, were assembled from an IDR-(Inverted Direct Response)-HMQC-TOCSY spectrum with an 18 msec mixing time. The protonated carbon fragments were conveniently located and oriented in the structural framework of **4** on the basis of observed nOe's from the NH, the $N-CH_3$, and methylene groups, as well as long-range het-

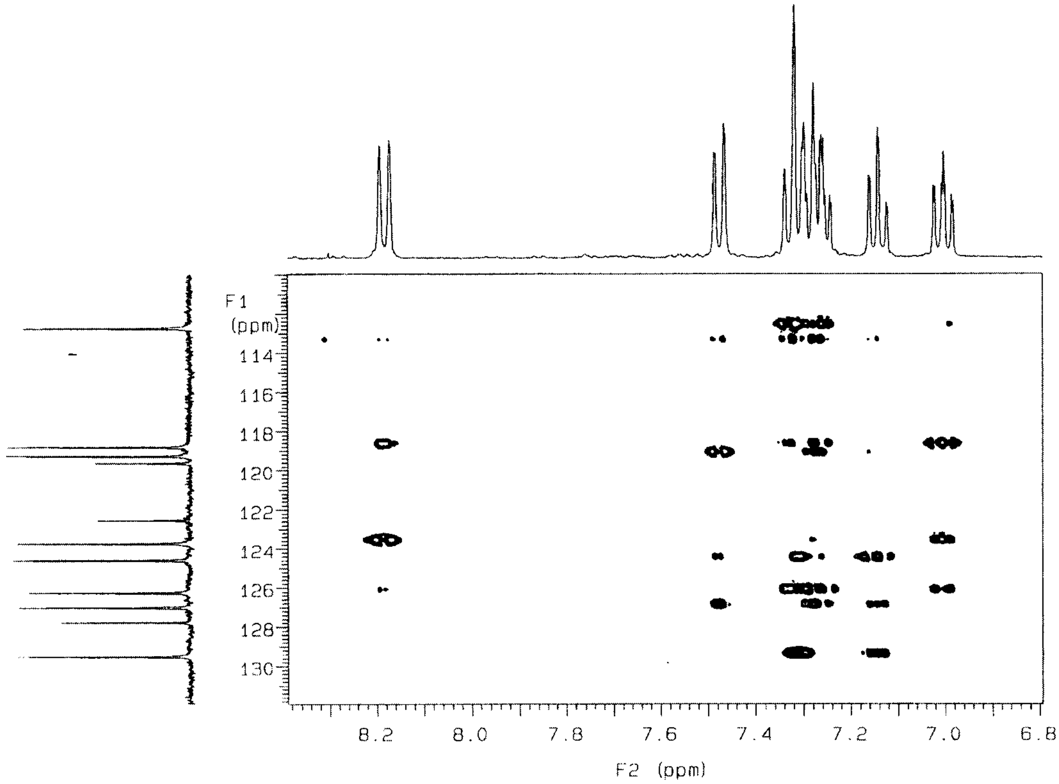


Figure 2. IDR-(Inverted Direct Response)-HMQC-TOCSY [9] spectrum of 4 recorded in d_6-DMSO at 500 MHz with an 18 msec isotropic mixing period. Direct responses are plotted in red and are negatively phased; relayed responses are positively phased and are presented as black contours.

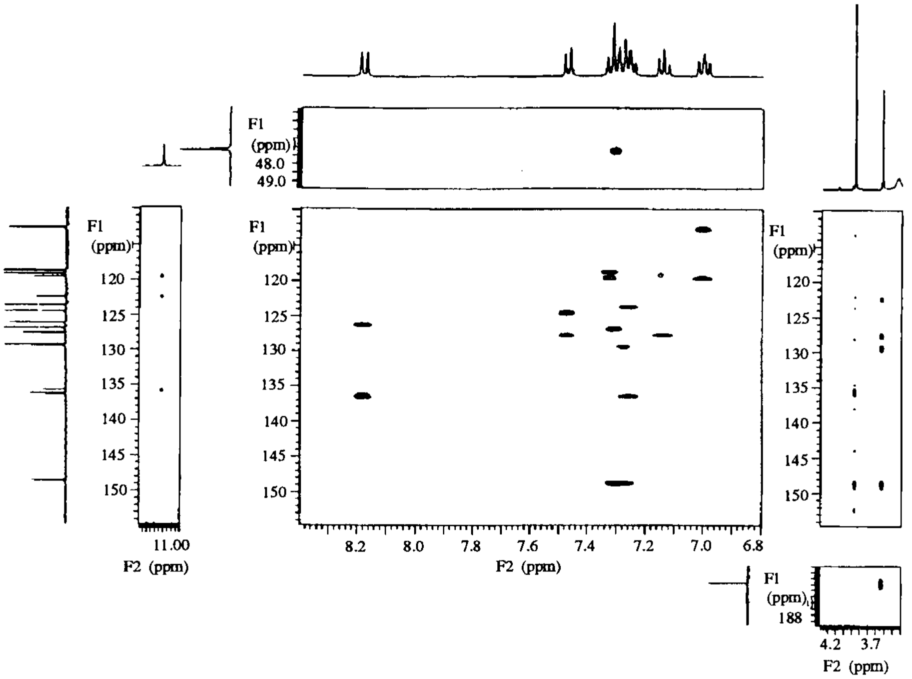
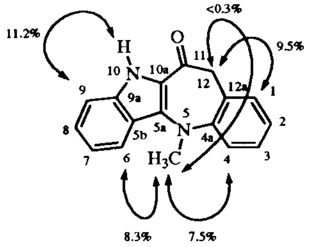


Figure 3. HMBC long-range heteronuclear shift correlation spectrum recorded in d_6-DMSO at 500 MHz [10]. Five individual panels are shown. The pair of panels to the right show the four responses from the *N*-methyl and 12-methylene proton resonances, to the carbonyl resonance (bottom right), and to the aromatic carbon resonances (top right). The center panels show responses from aromatic protons to various protonated and quaternary aromatic carbons (bottom center) and from the H1 aromatic proton to the 12-methylene carbon (top center). The final panel (left) shows correlations from the 10NH resonance to various aromatic carbons. Segments of the proton spectrum are plotted above appropriate panels or pairs of panels. Likewise, segments of the carbon reference spectrum are plotted vertically flanking appropriate panel segments. The experiment was optimized for a 10 Hz long-range coupling. Regions of the spectrum not shown contained no responses.

eronuclear correlations from an HMBC spectrum [10]. Pertinent nOe correlations are shown in **5** and included a <0.3% nOe between the methylene singlet and the *N*-methyl group; long-range correlations in the HMBC spectrum are shown in Figure 3.



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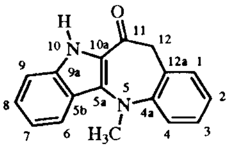
Long-range heteronuclear correlations observed in the HMBC spectrum of **4** assigned the seven quaternary carbon resonances and confirmed the orientation of the two contiguous protonated carbon fragments. Long-range HMBC correlations to the quaternary carbons and key positions are illustrated by **6**; a complete summary of all connectivities observed in the HMBC spectrum is given in Table 1.

Table 1

500 MHz ^1H and 75 MHz ^{13}C Resonance Assignments of Homocryptolepinone (**4**) in d_6-DMSO and Long-range Heteronuclear Correlations Observed in the 500 MHz HMBC Spectrum of **4**



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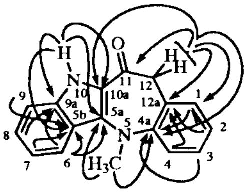
Position	^1H	^{13}C	Long-Range HMBC Correlation from Proton to Carbon Indicated
1	7.30	129.6	C3, C4a, C12
2	7.14	124.6	C4, C12a
3	7.25	126.3	C1, C4a
4	7.47	119.3	C2, C12a
4a	—	148.9	—
5-Me	3.97	41.2	C4a, C5a
5a	—	135.9	—
5b	—	119.6	—
6	8.18	123.8	C5a, C5b, C8, C9a
7	7.00	118.8	C5b, C9
8	7.27	127.1	C6, C9a
9	7.33	112.8	C5b, C7
9a	—	136.5	—
10-NH	11.22	—	C5a, C5b, C9a, C10a
11	—	182.9	—
12	3.62	47.7	C1, C4a, C10a, C11 (C=O), C12a

The carbonyl resonance had long-range correlations to only the methylene singlet, which was, in turn, strongly long-range coupled to both C1 and C4a, and more weakly to the C12a quaternary carbon resonance *via* two-bonds. The methylene singlet was also long-range coupled to a quaternary carbon resonating at 122.6 ppm, whose only other long-range correlation was to the NH proton resonating at 11.22 ppm. The latter correlation assigned the C10a quaternary carbon resonance. The correlations observed in the HMBC spectrum for the methylene singlet, in conjunction with a 9.5% nOe observed between the methylene and the proton resonating at 7.29 ppm, required the methylene to be at the 12-position of an indolobenzazepine carbon skeleton. The observed correlations also irrefutably located the carbonyl group at the 11-position of the indolobenzazepine skeleton. Hence, homocryptolepinone (**4**), an indolo[3,2-*b*]benzazepin-5*H*-4-one, had a novel and unprecedented skeleton in the growing family of alkaloids isolated from *C. sanguinolenta*.



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Molecular Modeling.

The structure of homocryptolepinone (**4**) was modeled in an effort to better visualize the structure and to account for the observed <0.3% nOe between the 12-methylene and *N*-methyl substituents when the latter was irradiated. A conformational search of homocryptolepinone (**4**) was performed with the Monte Carlo search options in MacroModel [6,11,12]. The MM3* force field was used with additions and point charge electrostatics [13]. Two conformations were found, one 6.6 KCal lower than the other. The low energy conformation was further refined using *ab initio* quantum mechanics. The MacroModel structure was geometry-optimized using the 3-21G split-valence basis set in Gaussian 92 to yield the minimized structure of **4** is shown in Figure 4. Key distances determined from the minimized structure were: $12CH_2$-H1 2.36Å; NMe-H4, 2.41Å; NMe-H6, 2.29Å; NMe-$12CH_2$, 3.54Å; and NH-H9, 2.85Å. Based on the observed distances between substituents flanking key structural features, the relative nOes were reasonable as was the observation of the weak nOe between the NMe and the 12-methylene resonances.

As shown by the structure, homocryptolepinone (**4**) was folded about an axis passing through the NMe and 12-

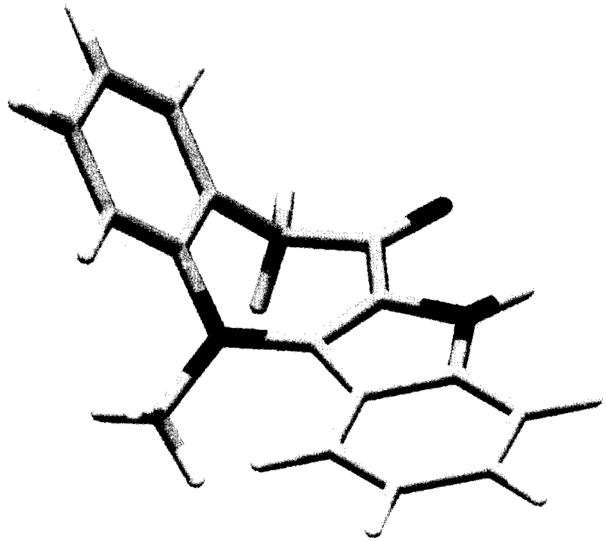

Figure 4. Energy minimized structure of homocryptolepinone (**4**) obtained by molecular modeling.

methylene. The dihedral angle of the planes comprised of the phenyl of the benzazepine, the NMe, and 12-methylene of the molecule and the indole, the NMe, and 11-carbonyl was 51.4°.

Conclusions.

Homocryptolepinone (**4**), an indolobenzazepinone, represents a new member of the *Cryptolepis* family of alkaloids. The indolobenzazepinone skeleton of **4** structurally resembles the bulk of the benzpyrrolizinobenzazepine (**3**) portion of the cryptospirolepine framework (**1**). Biogenetically, at this point we can assume that cryptospirolepine (**1**) may be assembled from cryptolepine or an appropriately 11-substituted congener and homocryptolepinone (**4**), through reaction at the 11-position of the former, or its 11-substituted analog, and at the NH and 11-carbonyl moieties of homocryptolepinone (**4**).

EXPERIMENTAL

The isolation of homocryptolepinone (**4**) was described above. The isolated 3.5 mg sample of the alkaloid was dissolved in 130 μl of 99.96% d_6-DMSO (Cambridge Isotope Laboratories) and transferred to a straight 3mm nmr tube (Wilmad) using a flexible Teflon needle. The tube was sealed after transfer.

Positive and negative ion apci-ms were recorded using the residue from the nmr sample preparation dissolved in methanol. The data were acquired using a Finnegan 4500 mass spectrometer with methane as the ionization gas.

The nmr spectra were recorded using a variety of spectrometers and probes as follows: the proton reference and HMQC [8] spectra were recorded using a Varian Unity 500 spectrometer equipped with a Nalorac Z•SPEC® micro inverse-detection (MID-500-3) probe. The latter was acquired as 2048 x (96 x 2) hypercomplex files with 8 transients/t_1 increment, a null interval of 0.4 sec, and a 1.315 sec interpulse delay. Spectral widths were 6358.3 and 3397.4 Hz in F_2 and F_1, respectively. Fixed delays were optimized as a function of $^1J_{CH}$ = 165 Hz. The ^{13}C reference spectrum was acquired using the same sample on a Varian VXR300S spectrometer equipped with a Nalorac Z•SPEC 5 mm quad-nucleus probe. The 75 MHz ^{13}C spectrum plotted flanking the various inverse-detected spectra was acquired in 52992 transients. The IDR-HMQC-TOCSY [9] and HMBC [10] spectra were acquired using a Varian Unity 400 spectrometer equipped with a Nalorac Z•SPEC MID-400-3 micro inverse probe. The former was acquired using an 18 msec isotropic mixing period for which pulse power was attenuated by 9 dB (24.3 μsec at tpwr = 48 vs. 8.9 μsec at tpwr = 57). The mixing interval was flanked by 2 msec trim pulses. The data were acquired as 2048 x (64 x 2) hypercomplex files. A total of 128 transients were accumulated/t_1 increment. The null interval was 0.4 sec; the interpose delay was 1.2 sec. Spectral widths were 5000 and 3142.4 Hz in F_2 and F_1, respectively. Finally, the HMBC data were acquired as 4096 x (384 x 2) hypercomplex files with 64 transients accumulated/t_1 increment. The experiment was optimized for an assumed long-range coupling of 10 Hz (50 msec). The interpulse delay was 1.8 seconds. Spectral widths were 5000 and 16091.6 Hz in F_2 and F_1, respectively.

Nuclear Overhauser difference spectra were recorded as 8K files at 400 MHz using interleaved data acquisition giving approximately a 1 second acquisition time. Irradiations were performed on-resonance or 3500 Hz downfield of the transmitter for 6 seconds. Blocks of eight acquisitions were acquired on- and off-resonance in a repetitive fashion to afford a total of 64 transients/irradiation frequency. Difference spectra were computed by subtracting the off-resonance reference irradiation fid from the on-resonance irradiation fid followed by zero-filling to 64 K points and exponential multiplication with a 2.5 Hz line broadening.

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Dr. Martin, please include another copy of both the black and colored portion of Figure 2. Make sure they are identical in size so we can scan them in and merge them correctly. Also, Figure 4 will be printed in color when published.