The Isolation and Structure Determination of Cryptomisrine, A Novel Indolo[3,2-*b*]quinoline Dimeric Alkaloid from *Cryptolepis sanguinolenta*

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Dedicated to the memory of Professor Nicholas Alexandrou

The isolation and structure determination of cryptomisrine, a novel indolo[3,2-*b*]quinoline dimeric alkaloid obtained from extracts of the roots of the Ghanaian medicinal plant *Cryptolepis sanguinolenta* is reported. The structure determination was made *via* a consideration of the spectral data, including uv, ir, nmr, and mass spectra. In particular, one-dimensional proton/carbon nmr, one-dimensional nOe difference nmr, and a series of homonuclear (COSY) and inverse-detected heteronuclear two-dimensional (HMQC, HMBC) experiments were utilized, as well as high resolution FABMS. Cryptomisrine is most unusual in that its two monomeric parts apparently exist in such a C_2 symmetric environment that only one set of proton and carbon nmr resonances are observed. Cryptomisrine is the first example of a dimeric indolo-[3,2-*b*]quinoline alkaloid to have been isolated from nature.

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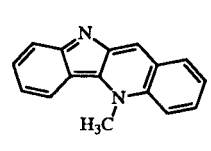
Introduction.

Cryptolepis sanguinolenta (Lindl.) Schlechter (*Asclepiadaceae*), a shrub indigenous to West Africa, has long been employed by Ghanaian traditional healers as a medicinal plant for use in the therapy of various fevers, including malaria [1]. The roots of this plant have been used in the form of a decoction in the clinical therapy of malaria, urinary tract infections, and upper respiratory tract infections at the Centre for Scientific Research into Plant Medicine at Mampong-Akwapim, Ghana since 1974 [1]. The root has been used in the Congo as a bitter stomachic [2], and in Nigeria in the therapy of colic, rheumatism, and urogenital infections [3].

In our continuing studies on the alkaloidal constituents of the roots of this plant, we have recently reported on the isolation and detailed nmr spectroscopic analysis of the indoloquinoline alkaloids cryptolepine (**1**) [4] and quindoline (**2**) [5]. In addition, we have also reported the isolation and elucidation of structure of cryptospirolepine (**3**) [6], a novel spirononacyclic indoloquinoline alkaloid that may be biogenetically derived from cryptolepine (**1**) [4]. Most recently we have described the determination of



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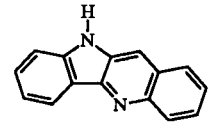

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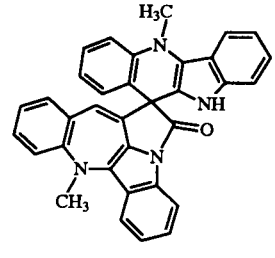

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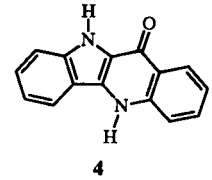

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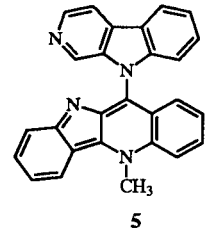

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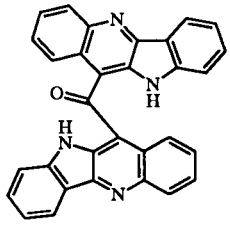
structure of two additional new alkaloids from this plant, namely the quindoline derivative, quindolinone (**4**) [7], and the novel indoloquinoline-β-carboline dimer cryptolepicarboline (**5**) [8].

The purpose of this present paper is twofold: first, to report the isolation of 15 additional alkaloids from extracts of the roots of this plant; and second, to describe the elucidation of structure of one of these alkaloids, a novel C-11 indolo[3,2-*b*]quinoline dimer that we have named cryptomisrine (**6**). Seven of the 15 additional alkaloids that have been isolated are novel, not having been isolated previously from nature nor having been described as a synthetic product, while the remaining 8 alkaloids are of an incompletely or partially determined structure at the present time.



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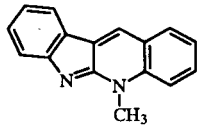
Isolation.

The dried and powdered roots of *C. sanguinolenta* (Lindl.) Schlechter were extracted by percolation with 95% ethanol. The extract was concentrated under reduced pressure to a viscous residue that was stirred with a dilute solution of citric acid and filtered. The insoluble portion was treated with ether, filtered, and the filtrate repartitioned with citric acid solution. The ether was evaporated to leave a residue of nonbasic compounds (Fraction A); while the citric acid solutions were pooled, alkalinized with ammonium hydroxide, and extracted with chloroform. Evaporation of the chloroform afforded a violet residue of basic compounds (Fraction B). Chromatography of Fraction B over neutral alumina (Column A) in petrol-chloroform (1:2), and successive elution with petrol-chloroform mixtures, chloroform, and chloroform-methanol mixtures afforded 15 fractions.

Fractions 1 and 2 (Column A) were combined and chromatographed over silicic acid (Column B) in chloroform. Elution with chloroform and chloroform-methanol mixtures afforded 11 fractions. Preparative hplc of fraction 1 (Column B) gave small amounts of two incompletely characterized alkaloids, designated as Alkaloid **A** and Alkaloid **B**. Preparative hplc of fraction 3 (Column B) gave quindoline (**2**) and cryptomisrine (**6**), the latter being obtained after a second preparative hplc purification. The structure determination of cryptomisrine (**6**) is the subject of subsequent discussion in this paper. Preparative hplc of fraction 4 (Column B) yielded additional amounts of quindoline (**2**), as well as three novel C-11 oxygen-substituted indolo[3,2-*b*]quinoline alkaloids, quindolinone (**4**) [7], cryptolepinone, and 11-methoxyquindoline. The determination of structure of the latter two alkaloids will be described elsewhere in due course. Chromatography of fraction 7 (Column B) over silicic acid in chloroform (Column C) and elution with chloroform-methanol mixtures afforded six fractions. Combination of fractions 2 and 3 (Column C) followed by preparative hplc yielded cryptotackieine (**7**) [9], a novel indolo[2,3-*b*]quinoline alkaloid. Preparative hplc of fraction 8 (Column B) afforded additional amounts of cryptotackieine (**7**), in addition to an incompletely characterized alkaloid that has been designated as Alkaloid **C**. Evaporation of fraction 9 (Column B) afforded an orange residue of another incompletely characterized alkaloid, this being designated as Alkaloid **D**.



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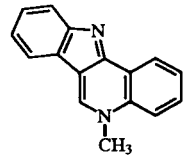

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Fraction 3 (Column A) was subjected to preparative tlc over silica gel, affording additional amounts of quindoline (**2**) and Alkaloid **D**, as well as a mixture of several other alkaloids (Mixture A). Preparative tlc of fraction 4 (Column A) likewise furnished additional amounts of quindoline (**2**), as well as a separable mixture (*via* differential solvent solubilities) of the incompletely characterized alkaloids, Alkaloid **D** and Alkaloid **E**. In addition, a fraction was obtained that contained a mixture of a few other alkaloids (Mixture B). Combination of Mixture A and Mixture B, followed by preparative hplc, afforded Alkaloid **A**, cryptomisrine (**6**), an additional amount of Alkaloid **B**, and still another unidentified alkaloid, Alkaloid **F**.

Fraction 7 (Column A) was subjected to preparative hplc, yielding four major alkaloid fractions. Additional preparative hplc of each of these fractions furnished the following alkaloids: cryptosanguinolentine, a novel indolo[3,2-*c*]quinoline alkaloid (**8**) [9a], whose structure determination was described along with that of cryptotackieine [9]; Alkaloid **G**; cryptolepicarboline (**5**), a novel indoloquinoline-β-carboline dimeric alkaloid whose structure has been recently reported [8]; and a sixth unidentified alkaloid, designated as Alkaloid **H**.

Treatment of fraction 8 (Column A) with absolute ethanol furnished cryptospirolepine (**3**) [6], while preparative hplc of fraction 9, followed by column chromatography, afforded additional amounts of cryptosanguino-

lentine. Treatment of fraction 10 with acetone gave an insoluble portion [identified as cryptolepine (**1**)], and a soluble portion. Preparative hplc of the soluble portion, followed by column chromatography, provided additional amounts of cryptosanguinolentine (**8**) and Alkaloid **G**.

Finally, treatment of fractions 11 and 12 (Column A) with acetone gave a crystalline mass, which was mainly cryptolepine (**1**). Preparative hplc of this mass afforded cryptolepine (**1**) and cryptolepinone, the latter of which is a new C-11 oxygen-substituted indolo[3,2-*b*]quinoline alkaloid, whose structure determination will be described in due course.

Structure Elucidation.

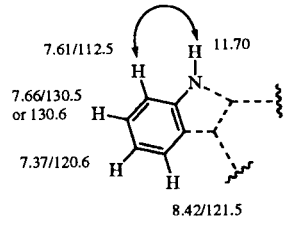
Cryptomisrine (**6**) was isolated as an orange residue (1.4 mg) after column chromatography of the alkaloid fraction (Fraction B), which was followed by preparative hplc. The uv spectrum of the alkaloid was characterized by maxima at 227 nm (log λ 4.85), 273 (4.90), 348 (4.19), 368 (4.18), and 459 (3.94), and was consistent with that of an indolo[3,2-*b*]quinoline alkaloid of the quindoline-type (**2**) [5,10]. Addition of 0.1*N* methanolic hydrochloric acid produced a bathochromic shift, with maxima appearing at 222 nm (log λ 4.90), 275 (4.86), 374 (4.48), and 475 (3.89). There was no change in the spectrum after the addition of 0.1*N* methanolic sodium hydroxide. The FT-IR spectrum (film) displayed a carbonyl absorption at 1727 cm^{-1}, and characteristic aromatic absorbances at 1614 and 1490 cm^{-1}. The EIMS showed the molecular ion (M^+) at m/z 462 (27%), with a significant fragment ion at m/z 245 (19%). The FABMS was supportive and displayed the $(M+H)^+$ ion at m/z 463 (100%). A high resolution EIMS displayed the molecular ion at m/z 462.1499, which was consistent with the empirical formula $C_{31}H_{18}ON_4$ (calculated 462.1497).

The proton nmr spectrum of cryptomisrine (**6**) was recorded at 400 MHz in 160 µl of 99.96% d_6-DMSO in a Nalorac Z•SPEC™ MID-400-3 micro inverse-detection probe. The proton spectrum was relatively congested but did clearly demonstrate the presence of two four-spin systems analogous to those found in cryptolepine (**1**) [4] and quindoline (**2**) [5]. In addition a broad one-proton signal was observed downfield at 11.70 ppm (typical of NH resonance in the spectrum of quindoline (**2**) [5]), the absence of *N*- and/or *O*-methyl signals was conspicuous. Furthermore, the absence of the characteristic low field singlet assignable to the H-11 proton of the indoloquinoline alkaloids cryptolepine (**1**) [4] and quindoline (**2**) [5] was also apparent [8]. The H-11 proton is typically the lowest field aromatic proton in the proton nmr spectrum of the indoloquinoline alkaloids [4,5,7].

A 100 MHz ^{13}C-nmr spectrum of cryptomisrine (**6**) was recorded using a Nalorac Z•SPEC MD-400-3 microdual probe. The spectrum exhibited 15 carbon resonances in the aromatic/vinylic region, plus an additional carbon resonance downfield at 195.6 ppm consistent with a carbonyl carbon between an aromatic ring and another aromatic or aliphatic entity (*e.g.* benzophenone at 195.2 ppm and acetophenone at 196.9 ppm [11]).

The direct proton-carbon chemical shift correlations were established from an HMQC [12] spectrum; long-range correlations were obtained from an HMBC [13] spectrum optimized for a long-range coupling of 10 Hz (50 msec).

The proton doublet resonating at 7.61 ppm corresponds to one of the terminal spins of the indolyl four-spin system, while its directly coupled carbon (112.5 ppm, HMQC) had a chemical shift characteristic of the C-9 of cryptolepine (**1**) [4] and quindoline (**2**) [5], and thereby provided a starting point for the structure elucidation. The COSY spectrum of the aromatic region provided connectivity information that were used to sequence the two four-spin systems. *Via* the utilization of the doublet resonating at 7.61 ppm as a point of departure, the assembly of the indole-derived four-spin system was straight-forward. A cross peak was observed (COSY) correlating the doublet resonating at 7.61 ppm to the the multiplet resonating at 7.66 ppm, the latter correlated, in turn, to the multiplet resonating at 7.37 ppm, which on the basis of chemical shift must be the proton para to the indole annular nitrogen. Finally, the proton resonating at 7.37 ppm displayed a correlation to the doublet resonating at 8.42 ppm. The proton at 11.70 ppm was assigned as the indole NH; the assignment supported by a nOe observed between the NH proton resonance at 11.70 ppm and the proton doublet resonating at 7.61 ppm. The corresponding carbon resonances of the indole-derived four-spin system were readily assigned using the HMQC data, as shown by partial structure **9**.



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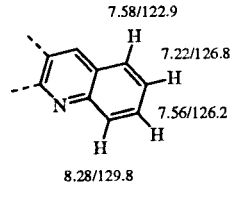

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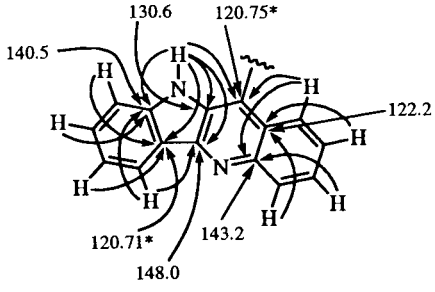
Similarly, the COSY spectrum was then utilized to assemble the proposed quinolinyl-derived four-spin system. Starting with the doublet resonating at 8.28 ppm, a correlation was observed to the multiplet resonating at 7.56 ppm. The proton at 7.56 ppm was correlated to the multiplet resonating at 7.22 ppm, which, in turn, was finally coupled to the doublet resonating at 7.58 ppm. This connectivity network is shown by partial structure **10**.

The orientation of the proposed quinoline-derived portion of the molecule was achieved using the long-range

connectivities observed in the HMBC spectrum. Correlations were observed from the protons resonating at 7.58 ppm and 7.56 ppm to a quaternary carbon resonating at 143.2 ppm, allowing the assignment of the quaternary carbon resonating at 143.2 ppm as C-4a, which was a reasonable chemical shift for a carbon bearing an annular nitrogen [11]. Accordingly, the doublet resonating at 7.58 ppm and the multiplet resonating at 7.56 ppm were assigned as H-1 and H-3, respectively. Long-range three-bond connectivities were also observed from the protons resonating at 8.28 and 7.22 ppm to the quaternary carbon resonating at 122.2 ppm. Thus, the quaternary carbon resonating at 122.2 ppm was assigned as C-11a and the protons resonating at 7.22 ppm and 8.28 ppm were obviously assigned as H-2 and H-4, respectively. The quaternary carbon resonating at 120.7 ppm showed a long-range connectivity to the H-1 doublet resonating at 7.58 ppm and consequently may be assigned as C-11. These connectivities are shown by partial structure **11**.



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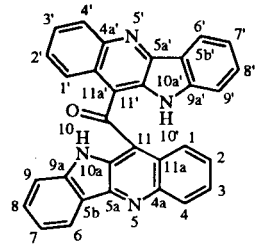
The two substructures were combined and the assignments were completed using the long-range heteronuclear shift correlations obtained from the HMBC spectrum (Figure 3).The indolyl NH, resonating at 11.70 ppm, exhibited three-bond connectivities to five quaternary carbons resonating at 120.71, 120.75, 130.5 or 130.6, 145.0, and 148.0 ppm. One carbon resonating at 120.7 ppm was previously assigned as C-11. The second quaternary carbon resonating at 120.7 ppm showed connectivities to the doublet resonating at 7.61 (H-9) and to the multiplet resonating at 7.37 ppm (H-7), and was thus assigned as C-5b. The carbon resonating at 148.0 ppm displayed a weak connectivity to the doublet resonating at 8.42 ppm (H-6) and was consequently assigned as C-5a. It should be noted, however, that the assignments of the quaternary resonances at 120.71 and 120.75 may be transposed because of digital resolution limitations imposed by the HMBC spectrum.

In addition to the weak coupling to the C-5a carbon resonating at 148.0 ppm, the proton resonating at 8.42 ppm (H-6) showed a three-bond coupling to two other carbon resonances. The first of these two carbons is the quaternary carbon resonating at 145.0 ppm which was assigned as C-9a, while the second resonated at either 130.5 or 130.6 ppm and was assigned to C-8. Again, an unequivocal assignment of C-8 could not be made because of digital resolution limitations of the 2D data.

Returning to the long-range correlations of the NH proton, couplings were observed to the quaternary carbon resonating at 130.5 or 130.6 ppm, and to the C-9a carbon resonance at 145.0 ppm. The carbon resonating at 130.5 or 130.6 ppm showed no other connectivities in the HMBC spectrum, and was thus assigned as C-10a. Long-range correlations to the quaternary carbons are illustrated by partial structure **11**. Proton and carbon chemical shifts assignments for cryptomisrine (**6**) and a complete summary of all the long-range connectivities are presented in Table 1.

Table 1

400 MHz ^{1}H and 100 MHz ^{13}C NMR Chemical Shift Data for Cryptomisrine (**6**) in d_6-DMSO and Long-Range Connectivities Observed in an HMBC Spectrum Optimized for 100 msec (5 Hz).



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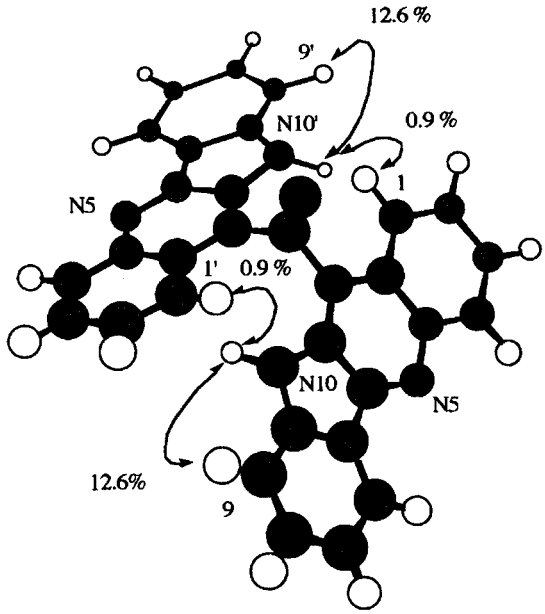
Position	Chemical Shift (δ) ppm ^{1}H	^{13}C	Long-Range Coupling Pathways [a]
1/1'	7.58	122.9	H-3
2/2'	7.22	126.8	H-4
3/3'	7.56	126.2	H-1
4/4'	8.28	129.8	H-2
4a/4'a	—	143.2	H-1, H-3
5a/5'a	—	148.0	NH, H-6 (weak)
5b/5'b	—	120.71 [b]	H-7, H-9, NH
6/6	8.42	121.5	H-8
7/7'	7.37	120.6	H-9
8/8'	7.66	130.5 [c]	H-6
9/9	7.61	112.5	H-7
9a/9'a	—	145.0	H-6, H-8, NH ($^2J_{CH}$)
10/10'	11.70	—	—
10a/10'a	—	130.6 [c]	NH ($^2J_{CH}$)
11/11'	—	120.75 [b]	H-1
11a/11'a	—	122.2	H-2, H-4
C=O	—	195.6	—

[a] From the proton specified in this column to the carbon located at the indicated position in the molecular structure. [b], [c] May be transposed.

The proton and carbon nmr data were indicative of a monomeric indoloquinoline structure, but the ms data (high and low resolution-M^+ 462 Da) mandated consideration of a dimeric compound. Consequently, cryptomisrine (**6**) must be dimeric, comprised of two 11-quindolinyl segments. The balance of the mass necessary to make the structure consistent with the mass spectral data is afforded

by the carbonyl resonating at 195.6 ppm, defining the structure as 11-(11-10*H*-indolo[3,2-*b*]quinolinoyl)-10*H*-indolo[3,2-*b*]quinoline. The lack of long-range correlation responses to the carbonyl is not particularly surprising, since the only possible couplings would be *via* $^4J_{CH}$ which are not often observed, particularly in non-rigid systems. Irradiation of the indole NH resonance at 11.70 ppm, in a one-dimensional nOe experiment, showed a 0.9% enhancement of the doublet resonating at 7.58 ppm, that had been previously assigned as the H-1 resonance.



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Consequently, the observed nOe must arise between the N10H moiety of the first indoloquinolinyl subunit and the H-1' resonance of the second or alternatively between N10H' and H1. Irradiation of the indoloquinolinyl NH resonances also gave a 12.6% nOe to the H9/9' resonances peri to the indolyl NH resonances. A computer-generated perspective drawing of the alkaloid cryptomisrine in a conformation of lowest energy illustrated the C_2 symmetric environment (**12**) of both monomeric halves of the structure allowing the rationalization of only one set of proton and carbon nmr resonances.

EXPERIMENTAL

General.

Diethyl ether and chloroform used in partitioning procedures were dried over anhydrous sulfate prior to filtration and evaporation *in vacuo* at 40°. Neutral alumina (Brockman Activity I) (80-200 mesh) (Fisher Scientific) and silicic acid (100 mesh) (Mallinckrodt) were used for column chromatography. Thin-layer chromatography was performed with 5 x 20 cm pre-Coated tlc sheets of silica gel 60 F_{254} (0.2 mm layer thickness, E. Merck). Preparative tlc was performed with 20 x 20 cm glass plates precoated with 1.0 mm silica gel 60 F_{254} (Analtech). The alkaloids were visualized *via* uv lamp and/or by dipping in Dragendorff Reagent [14]. The solvent system used for general analysis was chloroform-methanol-concentrated ammonium hydroxide (7:3:0.1) (solvent system A), while the systems used for preparative analysis included chloroform-methanol-concentrated ammonium hydroxide (9:1:0.1) (solvent system B) and benzene-acetone-concentrated ammonium hydroxide (15:10:0.1) (solvent system C). The hplc analysis was performed on one of three Waters Chromatography instruments. The hplc Instrument A was a Model 481 variable wavelength LC, with a Model 510 solvent delivery module, a model 590 programmable solvent delivery module, an automated gradient controller, a Model 440 fixed wavelength absorbance detector, a model U6K universal liquid chromatography injector fitted with a 2 ml sample loop, a model SE120 BBC Goerz metrawatt chart recorder, a Model 994 programmable photodiode array detector, and a model 5200 printer plotter. The hplc Instrument B was a Delta Prep 4000 preparative lc system, with a model 486 IEEE tunable absorbance detector fitted with a semipreparative cell (path length = 3 mm), a LAC/E module, with data collected on a MicroVAX 3100 using ExpertEase™ chromatography software. The hplc Instrument C was a Waters 625 LC System, with a model 486 IEEE tunable UV/Visible absorbance detector, a model 712 WISP, a LAC/E module, with data collected on a MicroVAX 3100 using ExpertEase™ chromatography software. Analytical columns included a high revolution reversed-phase Nova-Pak C_{18}60 Å 4 μm (3.9 mm x 150 mm) steel column (hplc Column A) or a high resolution reversed-phase Nova-Pak CN HP 4 μm (3.9 mm x 75 mm steel or 8 mm x 100 mm [semi-preparative] polymeric cartridge) column (hplc Column B).

Preparative columns included a high resolution 15-20 μ*M* preparative reversed-phase packing material (Waters Prep Bondapak C_{18} 125 Å) packed in three polymeric cartridge columns (40 mm x 100 mm) connected by Waters Segmented Column Technology (SCT) (hplc Column C); or a high resolution 6 micron preparative reversed-phase packing material (Waters Prep Nova-Pak HR C_{18} 60 Å) packed in one or two polymeric cartridge columns (40 mm x 100 mm or 25 mm x 100 mm or 8 mm x 100 mm [semipreparative]) connected by Waters Segmented Column Technology (SCT) (hplc Column D).

Plant Material.

The plant material used in this study consisted of the powdered, oven-dried (60°) roots of *Cryptolepis sanguinolenta* (Lindl.) Schlechter, known as Nibima by the populations endemic to Ghana. The plant was collected and subsequently identified by Professor Gilbert L. Boye of the Centre for Scientific Research into Plant Medicine, Mampong-Akwapim, Ghana, in 1990. A herbarium specimen is on deposit at the Centre.

Extraction and Fractionation.

Powdered, oven-dried roots (3.14 kg) were extracted by percolation with ethanol (55 l). The ethanol extract was concentrated to a viscous residue (870 g), which was stirred with citric acid (1%) (3.5 l). The citric acid-insoluble residue was treated with ether (1 l), filtered, and the filtrate partitioned with citric acid (1%) (1 l). The ether was evaporated to leave a brown residue (15.7 g) (Fraction A) of nonbasic compounds. The citric acid solutions were combined, alkalinized with ammonium hydroxide to *p*H 8-9, and extracted with chloroform (6 x 2 liters). The chloroform extracts were combined and evaporated to leave a violet residue (16.2 g) of basic compounds (Fraction B).

Chromatography of Fraction **B** (Column A).

Fraction **B** (16.2 g) was dissolved in chloroform (50 ml), adsorbed onto neutral alumina (20 g), and chromatographed over a column of neutral alumina (750 g) (Column A) prepared by sifting into petrol-chloroform (1:2). The polarity was gradually increased *via* the addition of chloroform and chloroform-methanol mixtures to afford 15 fractions, which were collected and subsequently combined according to tlc analysis (Solvent System A).

Chromatography of Fractions 1 and 2 from Column A (Column B).

Fractions 1 and 2 (Column A) were eluted with petrol-chloroform (1.7 l and 1 l, respectively) and combined because of their tlc similarity. The combined fraction (270 mg) was dissolved in chloroform (0.5 ml), and chromatographed over a column of silicic acid (100 g) (Column B), prepared from a slurry in chloroform. The polarity was gradually increased *via* the addition of methanol to afford 11 fractions, which were collected and subsequently combined according to tlc analysis (Solvent System A).

Isolation of Alkaloid **A** and Alkaloid **B**.

Fraction 1 (9.2 mg) (Column B) was dissolved in methanol (0.3 ml), and injected repeatedly onto hplc Column D (2 x 25 mm x 100 mm) using hplc Instrument A. Elution was *via* a gradient, using 40 m*M* ammonium acetate (*p*H adjusted to 7.0 with ammonium hydroxide) and methanol (1:1) for 105 minutes isocratic; followed by a 3:7 mixture for 50 minutes, and concluding with methanol alone for 15 minutes. The injection volume was 150 µl and the flow rate was 10 ml/minutes with uv detection at 340 nm. Thirteen fractions were collected, and each was subsequently concentrated *in vacuo*, diluted with water, alkalinized with ammonium hydroxide to *p*H 8-9, and extracted with chloroform. The chloroform extracts were evaporated to dryness to afford various fractions. Fractions 9 (1.1 mg) and 11 (1 mg) were chosen for purification, and fraction 9 was subjected to hplc purification using hplc Column B (semipreparative) and hplc instrument A. Fraction 9 was dissolved in methanol (150 µl) and injected onto Column B. The injection volume was 75 µl and the flow rate was 2 ml/minute with uv detection at 340 nm. Elution was isocratic, using 40 m*M* ammonium acetate (*p*H adjusted to 7.0 with ammonium hydroxide) and methanol (3:7) for 35 minutes. Alkaloid A was isolated as a yellow residue (<1 mg).

Fraction 11 (see above, described with fraction 9) was purified in an identical manner as fraction 9 to afford Alkaloid **B** as a yellow residue (<1 mg).

Isolation of Quindoline (**2**) and Cryptomisrine (**6**).

Fraction 3 (84.1 mg) (Column B) was dissolved in 0.01*M* ammonium acetate (*p*H 7.0)/methanol (1:1) (1 ml), and injected repeatedly onto hplc Column D (2 x 25 mm x 100 mm) using hplc Instrument A. Elution was *via* a gradient, using 40 m*M* ammonium acetate (*p*H adjusted to 7.0 with ammonium hydroxide) and methanol (1:1) for 140 minutes isocratic; followed by a 3:7 mixture for 70 minutes and concluding with methanol alone for 15 minutes. The injection volume was 100 µl and the flow rate was 10 ml/minute with uv detection at 320 nm. Ten fractions were collected, and each was subsequently concentrated *in vacuo*, diluted with water, alkalinized with ammonium hydroxide to *p*H 8-9, and extracted with chloroform. The chloroform extracts were evaporated to dryness to afford various fractions. Fraction 4 was isolated as a straw yellow solid residue (18 mg), identical as quindoline (**2**) by direct comparison (uv, ir, ^{1}H-nmr, ^{13}C-nmr) with literature data [5,9]. Fraction 8 (4.6 mg) was purified by preparative hplc under the same conditions as above. The sample was dissolved in dimethyl sulfoxide (200 µl), and injected in 50 µl aliquots. Elution was *via* a gradient, using 40 m*M* ammonium acetate (*p*H adjusted to 7.0 with ammonium hydroxide) and methanol (1:1) for 60 minutes, isocratic, followed by a 3:7 mixture for 70 minutes. Cryptomisrine (**6**) was obtained as an orange residue (1.4 mg) after the evaporation of fraction 4 (85-95 minutes. The tlc elution time) and purification *via* standard partitioning between ammonium hydroxide and chloroform. The tlc and hplc analysis indicated the presence of a single alkaloid; uv (methanol): λ_{max} 227 nm (log ε 4.85), 273 (4.90), 348 (4.19), 368 (4.18), and 459 (3.94); (0.1*N* Methanolic hydrochloric acid) 222 nm (log ε 4.90), 275 (4.86), 374 (4.48), and 475 (3.89); ir-ft (film): λ_{max} 1727, 1614, 1490, 1466, 1398, 1329, 1213, 1165, 1137, 1115, 804, and 748 cm^{-1}; ms: EI, M$^+$ m/z 462 (27%), with a significant fragment ion at m/z 245 (10); hrms: EI, M$^+$ m/z 462.1499 (calculated 462.1497 for $C_{31}H_{18}ON_4$); ms: FAB (M+H$^+$) m/z 463 (100%).

Isolation of Quindolinone (**4**), Cryptolepinone, Quindoline (**2**), and 11-Methoxyquindoline.

Fraction 4 (51.2 mg) (Column B) was dissolved in methanol (0.5 ml), and injected onto hplc Column D (2 x 25 mm x 100 mm) using hplc Instrument A. Elution was *via* a gradient, using 40 m*M* ammonium acetate (*p*H adjusted to 7.0 with ammonium hydroxide) and methanol (1:1) for 130 minutes, isocratic, followed by a 3:7 mixture for 90 minutes. The injection volume was 100 µl and the flow rate was 10 ml/minute with uv detection at 320 nm. Fourteen fractions were collected, and each was subsequently concentrated *in vacuo*, diluted with water, alkalinized with ammonium hydroxide to *p*H 8-9, and extracted with chloroform. The chloroform extracts were evaporated to dryness to afford various fractions. Quindolinone (**4**) (0.8 mg) was obtained as a yellow residue from fraction 2, while cryptolepinone was isolated as a lemon yellow residue (1.7 mg) from fraction 5. An additional amount (0.3 mg) of quindoline (**2**) was isolated from fraction 6, while 11-methoxyquindoline was obtained as a yellow residue (11 mg) from fraction 8.

Isolation of Cryptotackieine (**7**).

Chromatography of Fraction 7 (14.3 mg) (Column B) over silicic acid (100 g) in chloroform and elution with chloroform and chloroform-methanol mixtures gave six fractions. Fractions 2 and 3 were combined (2.9 mg), dissolved in methanol (150 µl), and injected onto hplc Column D (25 mm x 100 mm) using hplc Instrument A. Elution was *via* a gradient, using 40 m*M* ammonium acetate (*p*H adjusted to 7.0 with ammonium hydroxide) and methanol (1:1) for 30 minutes, isocratic, followed by a 2:3 mixture for 40 minutes. The injection volume was 75 µl and the flow rate was 10 ml/min, with uv detection at 330 nm. The fraction eluting between 53-57 minutes was collected, concentrated *in vacuo*, diluted with water, alkalinized with ammonium hydroxide to *p*H 8-9, and extracted with chloroform. The chloroform extract was evaporated to dryness to afford cryptotackieine (**7**) (<1.0 mg) as a yellow residue.

Isolation of Alkaloid **C** and Cryptotackieine (**7**).

Elution of Column B with chloroform-methanol (95:5) (2.2 liters) afforded fraction 8 as a brown residue (37 mg). Fraction 8 was dissolved in methanol (0.5 ml), and injected repeatedly onto hplc Column D (2 x 25 mm x 100 mm) using hplc Instrument A. Elution was *via* a gradient, using 40 m*M* ammonium acetate (*p*H adjusted to 7.0 with ammonium hydroxide) and methanol (1:1) for 84 minutes, isocratic; followed by a 3:7 mixture for 150 minutes. The injection volume was 100 µl and the flow rate was 9 ml/minute, with uv detection at 340 nm. Five fractions were collected, and each was subsequently concentrated *in vacuo*, diluted with water, alkalinized with ammonium hydroxide to *p*H 8-9, and extracted with chloroform. The chloroform extracts were evaporated to dryness to afford various fractions. Fractions 2 (1.9 mg) and 4 (2 mg) were chosen for purification, and fraction 2 was further purified *via* chromatography over neutral alumina (10 g) in chloroform to afford Alkaloid C as a yellow solid residue (1 mg).

Fraction 4 was subjected to hplc purification using hplc Column B (semipreparative) and hplc Instrument A. Fraction 4 (2 mg) was dissolved in methanol (200 µl) and injected onto Column B. The injection volume was 100 µl and the flow rate was 1.5 ml/minute, with uv detection at 350 nm. Elution was isocratic, using 40 m*M* ammonium acetate (*p*H adjusted to 7.0 with ammonium hydroxide) and methanol (1:1) for 35 minutes followed by a (3:7) mixture for 20 minutes. Cryptotackieine (**7**) was obtained as a yellow solid residue (<1 mg).

Isolation of Alkaloid **D**.

Continued elution of Column B with chloroform-methanol (95:5) (1 l) afforded fraction 9 as an orange residue (47 mg). The hplc and tlc analyses suggested chemical purity, and this compound was designated as Alkaloid **D**.

Isolation of Quindoline (**2**), Alkaloid **D** and Alkaloid **E**.

Continued elution of Column A with petrol-chloroform (1:2) (500 ml) and evaporation afforded Fraction 3 (50 mg) which was subjected to preparative tlc using Solvent System B. Additional amounts of quindoline (**2**) (2.2 mg) and Alkaloid **D** (21.7 mg) were isolated, as well as a mixture of 4 other alkaloids. Fraction 4 (Column A) (460 mg) was obtained *via* elution with petrol-chloroform (1:3) (1.6 liters), and was subjected to preparative tlc using Solvent System C. An additional amount of quindoline (**2**) (36.2 mg), as well as Alkaloid **D** (46.6 mg) and Alkaloid **E** (30 mg) were isolated. Furthermore, a mixture of 4 other alkaloids was obtained.

Isolation of Alkaloid **A**, Alkaloid **B**, Cryptomisrine (**6**), and Alkaloid **F**.

The mixtures of 4 alkaloids obtained from preparative tlc of both Fraction 3 (Column A) and Fraction 4 (Column A) were combined (30.5 mg) and subjected to repeated preparative hplc over hplc Column D using hplc Instrument A. Elution was *via* a gradient, using 40 m*M* ammonium acetate (*p*H adjusted to 7.0 with ammonium hydroxide) and methanol (1:1) for 110 minutes, isocratic; followed by a 3:7 mixture for 80 minutes and concluding with methanol alone for 30 minutes. The injection volume was 150 µl and the flow rate was 10 ml/minute, with uv detection at 330 nm. Fourteen fractions were collected, and each was subsequently concentrated *in vacuo*, diluted with water, alkalinized with ammonium hydroxide to *p*H 8-9, and extracted with chloroform. The chloroform extracts were evaporated to dryness to afford various fractions. Fractions 8 (1 mg), 10 (<1 mg), 12 (2 mg), and 13 (2 mg) were chosen for purification, and fraction 8 was subjected to hplc purification using hplc Column D (semipreparative) and hplc Instrument A. Fraction 8 was dissolved in methanol (150 µl) and injected onto Column D. The injection volume was 75 µl and the flow rate was 2 ml/minute with uv detection at 340 nm. Elution was isocratic, using 40 m*M* ammonium acetate (*p*H adjusted to 7.0 with ammonium hydroxide) and methanol (3:7) for 35 minutes. Alkaloid **A** was isolated as a yellow residue (<1 mg).

Alkaloid **B** was isolated as a yellow residue (<1 mg) from fraction 10 after similar treatment.

Fraction 12 was subjected to hplc purification using hplc Column D (25 mm x 100 mm) and hplc Instrument A. Fraction 12 was dissolved in DMSO (150 µl) and injected onto Column D. The injection volume was 150 µl and the flow rate was 10 ml/minute with uv detection at 340 nm. Elution was isocratic, using 40 m*M* ammonium acetate (*p*H adjusted to 7.0 with ammonium hydroxide) and methanol (1:1) for 140 minutes, followed by a 3:7 mixture for 50 minutes. Cryptomisrine (**6**) was isolated as an orange residue (<1 mg).

Fraction 13 was subjected to hplc purification using hplc Column D (8 mm x 100 mm) and hplc Instrument A. Fraction 13 was dissolved in DMSO (100 µl) and injected onto Column D. The injection volume was 100 µl and the flow rate was 10 ml/minute with uv detection at 340 nm. Elution was isocratic, using 40 m*M* ammonium acetate (*p*H adjusted to 7.0 with ammonium hydroxide) and methanol (1:1) for 75 minutes, followed by a 3:7 mixture for 50 minutes. Alkaloid **F** was isolated as a yellow residue (1.2 mg).

Isolation of Cryptosanguinolentine (**8**), Alkaloid **G**, Cryptolepicarboline (**5**), and Alkaloid **H**.

Elution of Column A with chloroform-methanol (99:1) (600 ml) and evaporation of the solvent afforded Fraction 7 (50 mg). Fraction 7 was dissolved in methanol (4 ml), and injected onto hplc Column D (2 x 40 mm x 100 mm) using hplc Instrument B. Elution was *via* a gradient, using water, 100 m*M* ammonium acetate (*p*H adjusted to 7.0 with ammonium hydroxide) and methanol (3:2:5) for 36 minutes, isocratic, followed by a 1:2:7 mixture for 50 minutes. The injection volume was 4 ml and the

flow rate was 50 ml/minute with uv detection at 310 nm for 15.5 minutes and then at 275 nm. Thirty six fractions were collected.

Fraction 2 (3.7 mg) was dissolved in methanol (150 µl), and injected onto hplc Column B (8 x 100 mm) using hplc Instrument A. Elution was *via* a gradient, using 40 m*M* ammonium acetate (*p*H adjusted to 7.0 with ammonium hydroxide) and methanol (7:3) to (2:3) over 30 minutes (linear gradient). The injection volume was 75 µl and the flow rate was 1.5 ml/minute with uv detection at 320 nm. Fraction 4 (8-11 minutes) was concentrated *in vacuo*, alkalinized with ammonium hydroxide to *p*H 8-9, and extracted with chloroform. The chloroform extracts were pooled and evaporated to dryness. Fraction 3 (4 mg) was dissolved in methanol (500 µl), and injected onto hplc Column B (8 x 100 mm) using hplc Instrument A. Elution was *via* a gradient, using 40 m*M* ammonium acetate (*p*H adjusted to 7.0 with ammonium hydroxide) and methanol (7:3) to (2:3) over 60 minutes (linear gradient). The injection volume was 15 µl and the flow rate was 1.5 ml/minute with uv detection at 330 nm. Fraction 4 (19-25 minutes) was concentrated *in vacuo*, alkalinized with ammonium hydroxide to *p*H 8-9, and extracted with chloroform. The chloroform extracts were pooled and evaporated to dryness to afford Alkaloid **G** as a violet residue (2.5 mg).

Fraction 6 (4 mg) was dissolved in methanol (200 µl), and injected onto hplc Column B (8 x 100 mm) using hplc Instrument A. Elution was isocratic, using 40 m*M* ammonium acetate (*p*H adjusted to 7.0 with ammonium hydroxide) and methanol (1:1) for 30 minutes. The injection volume was 20 µl and the flow rate was 1.5 ml/minutes with uv detection at 280 nm. Fraction 3 (21-29 minutes) was concentrated *in vacuo*, alkalinized with ammonium hydroxide to *p*H 8-9, and extracted with chloroform. The chloroform extracts were pooled and evaporated to dryness to afford cryptolepicarboline (**5**) [8] as a black residue (<<1 mg).

Fraction 19 (3 mg) was dissolved in methanol (100 µl), and injected onto hplc Column B (8 x 100 mm) using hplc Instrument A. Elution was *via* a gradient, using 40 m*M* ammonium acetate (*p*H adjusted to 7.0 with ammonium hydroxide) and methanol (7:3) to (2:3) over 30 minutes (linear gradient), and holding at (2:3) for 30 minutes. The injection volume was 15 µl and the flow rate was 1.5 ml/minute with uv detection at 280 nm. Fraction 3 (25.5-28 minutes) was concentrated *in vacuo*, alkalinized with ammonium hydroxide to *p*H 8-9, and extracted with chloroform. The chloroform extracts were pooled and evaporated to dryness to afford Alkaloid H as a yellow residue (<1 mg). The compound was further purified *via* chromatography over neutral alumina (10 g) in chloroform, and elution with chloroform.

Isolation of Cryptospirolepine (**3**).

Continued elution of Column A with chloroform-methanol (99:1) (700 ml) afforded Fraction 8 as a residue (40 mg). Treatment of this residue with absolute ethanol gave cryptospirolepine (**3**) (40 mg), mp >300°. Spectral data for this alkaloid have been reported in an earlier publication [6].

Isolation of Cryptosanguinolentine (**8**).

Continued elution of Column A with chloroform-methanol (99:1) (3 l) afforded Fraction 9 as a residue (100 mg). Fraction 9 was dissolved in methanol (1 ml), and injected repeatedly onto hplc Column D (2 x 25 mm x 100 mm) using hplc Instrument A. Elution was *via* a gradient, using 40 m*M* ammonium acetate (*p*H adjusted to 7.0 with ammonium hydroxide) and methanol (1:1) for 85 minutes, isocratic, followed by a 2:8 mixture for 50 minutes. The injection volume was 150 µl and the flow rate was 10 ml/minute with uv detection at 350 nm. Fraction 4 (12.5-30 minutes) was concentrated *in vacuo*, alkalinized with ammonium hydroxide to *p*H 8-9, and extracted with chloroform. The chloroform extracts were pooled and evaporated to dryness to afford cryptosanguinolentine (**8**) [9] as a greenish-yellow residue (17.2 mg). The alkaloid was further purified *via* chromatography over neutral alumina (10 g) in chloroform, with elution *via* chloroform-methanol (95:5).

Isolation of Cryptolepine (**1**), Cryptosanguinolentine (**8**), and Alkaloid **G**.

Continued elution of Column A with chloroform-methanol (99:1) (5.9 l) afforded Fraction 10 as a residue (360 mg). Treatment of this residue with acetone yielded a soluble portion (42 mg) and an insoluble portion (315 mg). The insoluble portion was identified as cryptolepine (**1**) while the soluble portion was submitted to preparative hplc. The acetone-soluble portion (42 mg) was dissolved in methanol (0.6 ml), and injected repeatedly onto hplc Column D (2 x 25 mm x 100 mm) using hplc Instrument A. Elution was *via* a gradient, using 40 m*M* ammonium acetate (*p*H adjusted to 7.0 with ammonium hydroxide) and methanol (1:1) for 55 minutes, isocratic; followed by a 1:9 mixture for 45 minutes. The injection volume was 120 µl and the flow rate was 10 ml/minute with uv detection at 350 nm. Fraction 5 (12-19 minutes) was concentrated *in vacuo*, alkalinized with ammonium hydroxide to *p*H 8-9, and extracted with chloroform. The chloroform extracts were pooled and evaporated to dryness to afford cryptosanguinolentine (**8**) [9] as a greenish-yellow residue (9 mg).

Fraction 8 (25-33 minutes) (2.5 mg) was further purified by semipreparative hplc. The fraction was dissolved in methanol (200 µl), and injected onto hplc Column B (8 mm x 100 mm) using hplc Instrument A. Elution was isocratic, using 40 m*M* ammonium acetate (*p*H adjusted to 7.0 with ammonium hydroxide) and methanol (1:1) for 30 minutes. The injection volume was 25 µl and the flow rate was 1.5 ml/minute with uv detection at 290 nm. Fractions 3 and 4 (10-13 minutes) were pooled, concentrated *in vacuo*, diluted with water, alkalinized with ammonium hydroxide to *p*H 8-9, and extracted with chloroform. The chloroform extracts were pooled and evaporated to dryness to afford a residue (1.5 mg). This semipreparative hplc purification step was repeated on this residue, and furnished Alkaloid **G** as a violet residue. The alkaloid was further purified *via* chromatography over silicic acid (10 g) in chloroform, and elution with chloroform and chloroform with an increasing amount of methanol.

Isolation of Cryptolepine (**1**) and Cryptolepinone.

Elution of Column A with chloroform-methanol (49:1) (2 l) and evaporation of the solvent gave Fraction 11 (2.05 g). Elution with chloroform-methanol (49:1-23:2) (8 l) and evaporation of the solvent provided Fraction 12 (5.5 g). Fractions 11 and 12 were combined because of a similar tlc pattern (Solvent System A), and the combined residue treated with acetone to produce a crystalline mass. Recrystallization of this mass from acetone provided a mass of yellow, needle-like crystals (6.17 g). Preparative hplc of a portion of this mass was accomplished *via*

dissolution in 400 m*M* acetate (*p*H 7.0)/methanol (1:1) (50 mg/ml), followed by chromatography over hplc Column C using hplc Instrument B. Elution was isocratic using water, 40 m*M* acetic acid (*p*H adjusted to 7.0 with triethylamine) and methanol (2:3:5). The injection volume was 75 ml and the flow rate was 50 ml/minute with uv detection at 310 nm for 15.5 minutes and then at 275 nm. One min fractions were collected for 77 minutes. Fraction 1 (1-33 minutes) was concentrated *in vacuo*, diluted with water, alkalinized with ammonium hydroxide to *p*H 8-9, and extracted with chloroform. The chloroform extract was evaporated to afford a violet residue. Treatment of this residue with acetone/methanol afforded bright yellow needles (5.44 g) of cryptolepine (**1**), mp 167-168°. Fraction 2 (34-64 minutes) was concentrated *in vacuo*, diluted with water, alkalinized with ammonium hydroxide to *p*H 8-9, and extracted with chloroform. The chloroform extract was evaporated to afford cryptolepinone as a straw yellow residue (160 mg).

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