# 11-Isopropylcryptolepine: A Novel Alkaloid Isolated from *Cryptolepis sanguinolenta* Characterized Using Submicro NMR Techniques

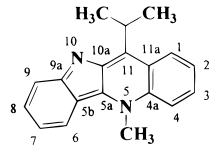
Chad E. Hadden,[†] Maged H. M. Sharaf,[‡] Jane E. Guido,[†] Russell H. Robins,[†] Albert N. Tackie,[§] Charles H. Phoebe, Jr.,[⊥] Paul L. Schiff, Jr.,[∥] and Gary E. Martin*[,†]

*Pharmacia & Upjohn, Pharmaceutical Development, Rapid Structure Characterization Group, Kalamazoo, Michigan 49001-0199, Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, Al-Azhar University, Nasr City, Cairo, Egypt, Centre for Scientific Research into Plant Medicine, Mampong-Akwapim, Ghana, Millipore−Waters Corporation, Milford, Massachusetts 01757, and Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, Pennsylvania 51261*

*Received June 24, 1998*

A new alkaloid has been isolated from extracts of the West African plant *Cryptolepis sanguinolenta* and identified by submicro NMR techniques as 11-isopropylcryptolepine (**1**). The unusual incorporation of the isopropyl group at the 11-position of the indolo[3,2-*b*]quinoline nucleus is suggestive of a mixed biosynthetic origin for the alkaloid.

Indoloquinoline alkaloids isolated from various *Cryptolepis* species have been the subject of considerable interest in recent years on the part of several research groups. Combined research efforts have led to the isolation and identification of a diverse assortment of alkaloids derived from three indoloquinoline skeletons. The vast majority of these alkaloids have been derived from the indolo[3,2-*b*]-quinoline system,[1−10] although there have also been examples of indolo[2,3-*b*]quinoline[11−15] and indolo[3,2-*c*]-quinoline[12,16] alkaloids reported. In addition, there have been several reports of indolo[3,2-*b*]benzazepine alkaloids,[9,17] several dimeric alkaloids incorporating indoloquinoline components,[11,18,19] and finally the unique spirononacyclic alkaloid cryptospirolepine.[20] We now report the isolation and structure elucidation of 11-isopropylcryptolepine (**1**) from *Cryptolepis sanguinolenta*.




**1**

## Results and Discussion

11-Isopropylcryptolepine (**1**) was isolated from the powdered, oven-dried (60 °C) roots of *Cryptolepis sanguinolenta* (Lindl.) Schlechter (Asclepiadaceae). The initial sample of the alkaloid, ≪1 mg, was isolated using the chromatographic procedures described previously.[18] At no point during the preparation of the crude plant material or the subsequent isolation was the sample in contact with any isopropyl-containing reagent or solvent. This eliminates the possible artifactual formation of 11-isopropylcryptolepine during the isolation scheme, as was the case with the dimeric alkaloid cryptoquindoline.[9,11]

The indolo[3,2-*b*]quinoline nucleus was inferred from preliminary micro (3 mm) NMR data. Aromatic resonances of the indolo[2,3-*b*]quinoline and indolo[3,2-*c*]quinoline nuclei exhibit unique chemical shifts[21] inconsistent with the data in hand. The data were consistent with and subsequently confirmed as an indolo[3,2-*b*]quinoline.[4]

The EIMS of **1** gave a parent ion at *m*/*z* 275, which is consistent with 11-isopropylcryptolepine, and a prominent ion for $M^+-CH_3$ at *m*/*z* 259. The loss of the N−Me substituent is common for cryptolepine and cryptolepinone.[22] Other fragment ions observed in the mass spectrum were consistent with the indoloquinoline nucleus. The HRMS of **1** gave an observed mass of 275.153 827 da, which agreed to within 3.6 ppm of the calculated mass for an empirical formula $C_{19}H_{19}N_2$ of 275.154 824 da.

A substantial portion (>50%) of the initial sample was consumed in the acquisition of preliminary MS data and the conversion of the isolated salt to the free base, leaving ca. 0.25 μmole (ca. 75 μg). After conversion to the free base, the sample changed color from yellow to a deep purple, similar to that of cryptolepine and consistent with the extended conjugation of the cryptolepine indolo[3,2-*b*]-quinoline nucleus.

After conversion to the free base, the small sample remaining required the utilization of submicro (1.7 mm) NMR techniques for the acquisition of both homonuclear and heteronuclear 2D NMR shift correlation data.[22,23] The aromatic region of the proton NMR spectrum of the free base was better resolved than the acid salt; homonuclear vicinal correlations of the aromatic spin-systems were successfully established from TOCSY data. ROESY data oriented the spin systems pairwise through the strong correlation from the *N*-methyl resonance to the flanking protons resonating at δ 8.51 (H-4) and 8.45 (H-6). The isopropyl methyl and methine resonances exhibited ROESY correlations to the doublet resonating at δ 8.70 (H-1), locating the isopropyl group at the 11-position. GHSQC data established the direct proton−carbon correlations in a few hours using submicro NMR techniques despite the small size of the remaining sample. These data were then supplemented with long-range $^1H-^{13}C$ correlations from an overnight GHMBC spectrum, which unequivocally established the structure of the alkaloid.

* To whom inquiries should be addressed: Pharmacia & Upjohn, Pharmaceutical Development, Rapid Structure Characterization Group, MS #4821-259-277, 7000 Portage Road, Kalamazoo, Michigan 49001-0199. Tel.: (616) 833-6283. Fax: (616) 833-6743. E-mail: gary.e.martin@am.pnu.com.

[†] Pharmaceutical Development, Pharmacia & Upjohn.
[‡] Department of Pharmacognosy and Natural Products Chemistry.
[§] Centre for Scientific Research into Plant Medicine.
[⊥] Millipore−Waters Corporation.
[∥] Department of Pharmaceutical Sciences.

10.1021/np980278t CCC: $18.00 
Published on Web 12/09/1998

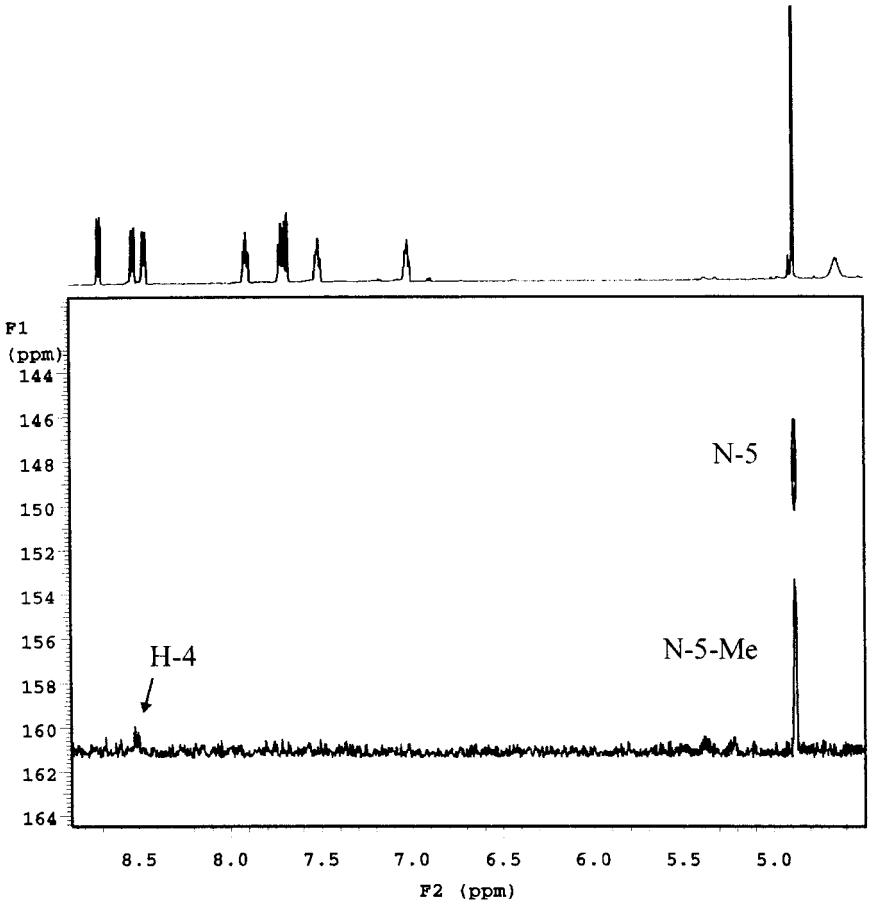



**Figure 1.** $^{1}H-^{15}N$ long-range GHMBC spectrum of ca. 0.25 $\mu$mol (ca. 75 $\mu$g) of **1** in 30 $\mu$L DMSO-$d_6$. The only response seen clearly in the contour plot is that of the N-5−Me protons to N-5. The $F_1$ trace, shown inside the box, shows a weak response from H-4 to N-5. The experiment was optimized for a 3.3-Hz long-range coupling,[14] and acquired as 2560 transients per $t_1$ increment. The hypercomplex data set, consisting of 512 × 40 points, was linear predicted to 1024 × 256 points, and then subjected to sinebell multiplication in $t_2$ and cosine multiplication in $t_1$ prior to tranformation. Total acquisition time was 77 h.

GHMBC correlations from the H-1 and the isopropyl methyl resonances allowed the assignment of the carbon resonating at $\delta$ 144.2 as C-11, fixing the attachment of the isopropyl group at the 11-position. GHMBC correlations from H-6 and H-8 confirmed the assignment of C-9a at $\delta$ 157.5. This chemical shift is consistent with the "imine-like" character of C-9a and is comparable to the $\delta$ 160.0 chemical shift of C-9a of cryptolepine.[4] The $\delta$ 157.5 chemical shift of C-9a also precludes the possibility of N-10 being protonated because C-9a of quindoline, which contains a protonated N-10 and no C-9a=N-10 carbon−nitrogen double bond, resonates upfield at $\delta$ 144.1.[15]

As long-range $^{1}H-^{15}N$ 2D heteronuclear shift correlation at natural abundance is becoming a viable experiment for small natural products structure determination,[24−26] an effort was made to acquire long-range $^{1}H-^{15}N$ GHMBC data for **1**. The first experiment was run overnight at 600 MHz on the acid salt sample using a 3-mm gradient inverse-micro NMR probe. The N-5 methyl protons exhibited a long-range correlation to the N-5 nitrogen resonating at $\delta$ 160.0, consistent with the N-5 chemical shift of cryptolepine at $\delta$ 154.7.[24] After conversion to the free base, a second experiment was acquired over a weekend using a submicro NMR probe at 600 MHz in an attempt to obtain the signal-to-noise ratio necessary to observe the correlations from the aromatic protons peri to both nitrogen resonances. The experiment was optimized for a 3.3-Hz coupling, based on the $^{1}H-^{15}N$ couplings in cryptolepine,[24] and is shown in Figure 1. Both the N-5−methyl protons and the H-4 proton correlated to the N-5 nitrogen, which shifted upfield by 12.2 ppm to $\delta$ 147.8, relative to the acid salt. Unfortunately, no correlations were seen to the N-10 nitrogen, although this was not entirely unexpected as there was only ca. 0.25 $\mu$mol (ca. 75 $\mu$g) of sample in the NMR tube. Corresponding long-range responses to peri nitrogens in other alkaloids have often proved difficult to observe even using generous samples.[24,26,27]

Overall, the elucidation of the structure of 11-isopropylcryptolepine was straightforward. There was excellent consistency relative to the reported chemical shifts of cryptolepine. Notable is the similarity of C-9a ($\delta$ 157.5) for 11-isopropylcryptolepine (**1**) compared to that of cryptolepine ($\delta$ 160.0),[4] which supports the imine-like character of the C-9a=N-10 exocyclic double bond. Likewise, the nitrogen chemical shift of the N-5 resonance at $\delta$ 147.8 is similar to that of the N-5 chemical shift in cryptolepine at $\delta$ 154.7.[24]

To date, there have been no reported studies of the biosynthesis of the indoloquinoline alkaloids. It is reasonable to assume, however, that the biosynthetic elaboration of the indoloquinoline nucleus utilizes both tryptophan and anthranilic acid pathways, which are known to be employed in the biosynthetic elaboration of indole and quinoline alkaloid skeletons, respectively. The presence of the isopropyl group makes it attractive to speculate that the biosynthesis may also involve the incorporation of an isoprene unit into the *Cryptolepis* alkaloids as a minor biosynthetic pathway.

**Table 1:** $^{1}H$, $^{13}C$, and $^{15}N$ Chemical Shifts for the Acid Salt and the Free Base of **1** in DMSO-$d_6$ at 600 MHz

| | acid salt | | free base | |
|---|---|---|---|---|
| position | $\delta^{1}H$ | $\delta$ $^{13}C$ and $^{15}N$ | $\delta^{1}H$ | $\delta$ $^{13}C$ and $^{15}N$ |
| 1 | 8.82 | 126.1 | 8.70 | 125.0 |
| 2 | 7.88 | 126.9 | 7.70 | 123.0 |
| 3 | 8.07 | 132.0 | 7.89 | 127.9 |
| 4 | 8.68 | 118.8 | 8.51 | 116.7 |
| 4a | | 135.8 | | 132.6 |
| 5 | | 160.0 ($^{15}N$) | | 147.8 ($^{15}N$) |
| 5-N−Me | 4.98 | 40.5 | 4.88 | 40.4 |
| 5a | | 137.9 | | 137.3 |
| 5b | | 114.0 | | 113.2 |
| 6 | 8.68 | 126.3 | 8.45 | 124.3 |
| 7 | 7.44 | 121.5 | 7.01 | 115.9 |
| 8 | 7.86 | 133.8 | 7.50 | 129.4 |
| 9 | 7.86 | 113.4 | 7.67 | 118.6 |
| 9a | | 145.5 | | 157.5 |
| 10 | | ($^{15}N$, N/A)[a] | | ($^{15}N$, N/A) |
| 10a | | 132.3 | | N/A |
| 11 | | 144.2 | | 144.2 |
| 11a | | 124.3 | | 121.4 |
| $^{i}$Pr−CH | 4.45 | 27.1 | 4.65 | 28.5 |
| $^{i}$Pr−$CH_3$ | 1.75 | 19.8 | 1.77 | 20.8 |

[a] Not applicable.

## Experimental Section

**General Experimental Procedures.** The structure of 11-isopropylcryptolepine (**1**) was determined via the concerted interpretation of a series of inverse-detected 2D NMR experiments. Preliminary studies were performed on a Varian Unity 400 MHz spectrometer equipped with Nalorac Z·SPEC MID-400-3 micro inverse and MC-400-3 micro carbon NMR probes. The studies on the free base and all of the long-range $^{1}H-^{15}N$ heteronuclear shift correlation experiments were performed on a Varian INOVA 600 MHz NMR spectrometer equipped with either a Nalorac Z·SPEC MIDTG-600-3 gradient-inverse 3-mm triple resonance probe or a Nalorac Z·SPEC SMIDG-600-1.7 submicro inverse-gradient NMR probe. For the initial studies on the Unity 400, the sample, existing as an acid salt, was dissolved in 150 $\mu$L of DMSO-$d_6$ 99.96% (Cambridge Isotope Laboratories) and transferred to a 3-mm Wilmad NMR tube via a Teflon needle using a Hamilton gastight syringe. After preliminary MS data acquisition, the sample was evaporated to dryness using a speed vac, redissolved in 30 $\mu$L 99.996% DMSO-$d_6$, followed by treatment with ammonia gas, to afford the free base. The sample was transferred to a 1.7-mm NMR tube using a Hamilton 10-$\mu$L syringe under a dry argon atmosphere and then sealed. The NMR tubes were made from Wilmad 1.7-mm precision glass tubing. The remaining sample was estimated to be ca. 0.25 $\mu$mol (ca. 75 $\mu$g), based on previous performance results from the SMIDG probe with samples prepared by serial dilution.[22,23,28] The gradient-optimized experiments performed on the free base of the alkaloid at 600 MHz included homonuclear TOCSY and ROESY experiments, GHSQC, a 10-Hz optimized $^{1}H-^{13}C$ GHMBC, and a 3.3-Hz optimized long-range $^{1}H-^{15}N$ GHMBC experiment. Pertinent experimental parameters are given in the figure captioning.

LRMS and HRMS measurements were made on a TSQ-7000 (Finnigan, Palo Alto, CA) and AutoSpec Q mass spectrometers (Micromass, Manchester, UK), respectively. LRMS data were acquired using $\mu$ESI by diluting 1 $\mu$L of the DMSO-$d_6$ solution used to prepare the NMR sample in 1 mL of 50:50 ACN−$H_2O$ containing 2% formic acid. HRMS measurements were performed by the simultaneous introduction of the reference material and sample using a dual channel micro-electrospray nebulizer built at Pharmacia & Upjohn. The instrument was tuned to a resolution of 10 000 (10% valley definition) using one of the monomers of poly(ethylene glycol). Data were acquired using a voltage scan framing the unknown between two poly(ethyelene glycol) reference peaks (256.17601 and 283.17568 da). The instrument was scanned over the mass range in 3 s in the multichannel analyzer mode accumulating 10 sub scans/individual scan.

**Plant Material and Isolation.** Refer to literature.[18]

**Compound 1.** For MS, see Results and Discussion. For NMR, refer to Table 1, Figure 1, and Supporting Information.

**Acknowledgment.** The authors would like to acknowledge Phil Sanders of Drug Metabolism Research, Pharmacia & Upjohn, for providing the HRMS data.

**Supporting Information Available:** GHSQC and GHMBC spectra of 11-isopropylcryptolepine (**1**, as the free base) acquired in DMSO-$d_6$ using 600 MHz submicro inverse-detection gradient (SMIDG) NMR probe technology (2 pages). Ordering information is given on any current masthead page.

## References and Notes

(1) Gellert, E.; Hamet, R.; Schlittler, E. *Helv. Chim. Acta* **1951**, *34*, 642−651.
(2) Dwuma-Badu, D.; Ayim, J. S. K.; Fiagbe, N. I. Y.; Knapp, J. E.; Schiff, P. L., Jr.; Slatkin, D. J. *J. Pharm. Sci.* **1978**, *67*, 433−434.
(3) Ablordeppey, S. Y.; Hufford, C. D.; Bourne, R. F.; Dwuma-Badu, D. *Planta Med.* **1990**, *56*, 416−417.
(4) Tackie, A. N.; Sharaf, M. H. M.; Schiff, P. L., Jr.; Boye, G. L.; Crouch, R. C.; Martin, G. E. *J. Heterocyclic Chem.* **1991**, *28*, 1429−1435.
(5) Spitzer, T. D.; Crouch, R. C.; Martin, G. E.; Sharaf, M. H. M.; Schiff, P. L., Jr. *J. Heterocyclic Chem.* **1991**, *28*, 2065−2070.
(6) Crouch, R. C.; Davis, A. O.; Spitzer, T. D.; Martin, G. E.; Sharaf, M. H. M.; Schiff, P. L., Jr.; Phoebe, C. H., Jr.; Tackie, A. N. *J. Heterocyclic Chem.* **1995**, *32*, 1077−1080.
(7) Sharaf, M. H. M. Ph.D. Dissertation; School of Pharmacy, University of Pittsburgh, Pittsburgh, PA, 1993.
(8) Cooper, M. M.; Lovell, J. M.; Joule, J. A. *Tetrahedron Lett.* **1996**, *37*, 4283−4286.
(9) Paulo, A.; Gomes, E. T.; Houghton, P. J. *J. Nat. Prod.* **1995**, *58*, 1485−1491.
(10) Sharaf, M. H. M.; Schiff, P. L., Jr.; Tackie, A. N.; Martin, G. E. *J. Heterocyclic Chem.* **1998**, *35*, in press.
(11) Cimanga, K.; De Bruyne, T.; Pieters, L.; Claeys, M.; Vlietinck, A. *Tetrahedron Lett.* **1996**, *37*, 1703−1706.
(12) Sharaf, M. H. M.; Schiff, P. L., Jr.; Tackie, A. N.; Phoebe, C. H., Jr.; Martin, G. E. *J. Heterocyclic Chem.* **1996**, *33*, 239−243.
(13) Alajarin, M.; Molina, P.; Vidal, A. *J. Nat. Prod.* **1997**, *60*, 747−748.
(14) Peczynska-Czoch, W.; Pognan, F.; Kaczmarek, L.; Boratynski, J. *J. Med. Chem.* **1994**, *37*, 3503−3510.
(15) Kamienska-Trela, K.; Kania, L.; Kaczmarek, L. *J. Mol. Spec.* **1995**, *347*, 467−476.
(16) Pousset, J.-L.; Martin, M.-T.; Jossang, A.; Bodo, D. *Phytochemistry* **1995**, 735−736.
(17) Sharaf, M. H. M.; Schiff, P. L., Jr.; Tackie, A. N.; Phoebe, C. H., Jr.; Davis, A. O.; Andrews, C. W.; Crouch, R. C.; Martin, G. E. *J. Heterocyclic Chem.* **1995**, *32*, 1631−1636.
(18) Sharaf, M. H. M.; Schiff, P. L., Jr.; Tackie, A. N.; Phoebe, C. H., Jr.; Johnson, R. L.; Minick, D.; Andrews, C. W.; Crouch, R. C.; Martin, G. E. *J. Heterocyclic Chem.* **1995**, *33*, 789−797.
(19) Sharaf, M. H. M.; Schiff, P. L., Jr.; Tackie, A. N.; Phoebe, C. H., Jr.; Howard, L.; Meyers, C.; Hadden, C. E.; Wrenn, S. K.; Davis, A. O.; Andres, C. W.; Minick, D.; Johnson, R. L.; Shockcor, J. P.; Crouch, R. C.; Martin, G. E. *Magn. Reson. Chem.* **1995**, *33*, 767−778.
(20) Tackie, A. N.; Boye, G. L.; Sharaf, M. H. M.; Schiff, P. L., Jr.; Crouch, R. C.; Spitzer, T. D.; Johnson, R. L.; Dunn, J.; Minick, D.; Martin, G. E. *J. Nat. Prod.* **1993**, *56*, 653−670.
(21) Sharaf, M. H. M.; Schiff, P. L., Jr.; Tackie, A. N.; Phoebe, C. H., Jr.; Martin, G. E. *J. Heterocyclic Chem.* **1993**, *33*, 239−243.
(22) Martin, G. E.; Guido, J. E.; Robins, R. H.; Sharaf, M. H. M.; Schiff, P. L., Jr.; Tackie, A. N. *J. Nat. Prod.* **1998**, *61*, 555−559.
(23) Martin, G. E.; Crouch, R. C.; Zens, A. P. *Magn. Reson. Chem.* **1998**, *36*, 551−557.
(24) Martin, G. E.; Crouch, R. C.; Sharaf, M. H. M.; Schiff, P. L., Jr. *J. Nat. Prod.* **1996**, *59*, 2−4.
(25) Martin, G. E.; Hadden, C. E. "Applications of $^{15}N$ NMR at Natural Abundance." Presented at the Varian User's Meeting, Palo Alto, CA, March 19−21, 1998.
(26) Hadden, C. E.; Martin, G. E. *J. Nat. Prod.* **1998**, *61*, 969−972.
(27) Martin, G. E.; Crouch, R. C.; Andrews, C. W. *J. Heterocyclic Chem.* **1995**, *32*, 1759−1766.
(28) Martin, G. E.; Robins, R. H. "Big Magnets and Teeny Little Probes." Presented at the 6th Annual Advances in NMR Applications Symposium, Monterey, CA, March 22 1998.

NP980278T