# *Notes*

## In Vitro and in Vivo Antiplasmodial Activity of Cryptolepine and Related Alkaloids from *Cryptolepis sanguinolenta*

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Three different extracts and four alkaloids from the root bark of *Cryptolepis sanguinolenta* have been assessed in vitro against *Plasmodium falciparum* D-6 (chloroquine-sensitive strain), K-1, and W-2 (chloroquine-resistant strains). Cryptolepine (**1**) and its hydrochloride (**2**), 11-hydroxycryptolepine (**3**), and neocryptolepine (**5**) showed a strong antiplasmodial activity against *P. falciparum* chloroquine-resistant strains. Quindoline (**4**) was less active. The highest activity was obtained with compound **1**. In vivo tests on infected mice showed that crytpolepine (**1**), when tested as its hydrochloride (**2**), exhibited a significant chemosuppressive effect against *Plasmodium berghei yoelii* and *Plasmodium berghei berghei*, while **1** had the same effect against *P. berghei yoelii* only. Compounds **3** and **4** did not show activity in this in vivo test system.

*Cryptolepis sanguinolenta* (Lindl.) Schlechter (Periplocaceae) is a climbing liana from Central and West Africa, where it is used by traditional healers in the treatment of infectious diseases, amoebiasis, and fever, including malaria.[1,2] In Central Africa (Zaire), the root bark of this medicinal plant is used as an aqueous macerate, while in West Africa (Ghana, Nigeria, and Senegal), the entire root is used as an aqueous decoction for the same purposes. Because of *C. sanguinolenta*'s use against malaria, we evaluated the activity of different root bark extracts and some of their constituents in vitro against *P. falciparum* and in vivo on mice infected with *P. berghei yoelii* and *P. berghei berghei*.

As shown in Table 1, all extracts from *C. sanguinolenta* root bark possessed a promising antiplasmodial activity against the *P. falciparum* chloroquine-sensitive strain D-6. The most active extract was the total alkaloid fraction (F3) with an $IC_{50}$ value of 47 ± 2.0 ng/mL. The antiplasmodial activity of the 80% EtOH extract (F2) ($IC_{50}$, 72 ± 1.5 ng/mL) was more pronounced than that of the aqueous extract (Zairean traditional remedy) ($IC_{50}$, 122 ± 1.9 ng/mL).

Concerning the two *P. falciparum* chloroquine-resistant strains K-1 and W-2, the results in Table 1 indicate that extract F3 displayed a strong antiplasmodial activity with $IC_{50}$ values of 42 ± 0.1 and 54 ± 0.7 ng/mL, respectively, which is significantly higher than that of chloroquine ($IC_{50}$, 72 ± 0.1 and 68 ± 0.1 ng/L against K-1 and W-2, respectively) when used as a reference. It was also observed that extract F2 ($IC_{50}$, 56 ± 0.1) exhibited a more pronounced antiplasmodial activity than chloroquine when tested against the K-1 strain, whereas the activity of the reference drug was higher than that of F2 against the W-2 strain. The aqueous extract (F1) was in all cases the less active extract.

**Table 1.** Antiplasmodial Activity ($IC_{50}$ ng/mL) of Extracts and Alkaloids from *Cryptolepis sanguinolenta* Root Bark Against Three *Plasmodium falciparum* Strains[a]

| | *P. falciparum* strain | | |
|---|---|---|---|
| extract/compd | D-6 | K-1 | W-2 |
| F1 | 122 ± 1.9 | 93 ± 0.1 | 168 ± 0.4 |
| F2 | 72 ± 1.5 | 56 ± 0.1 | 142 ± 0.3 |
| F3 | 47 ± 2.0* | 42 ± 0.1* | 54 ± 0.7 |
| cryptolepine (**1**) | 27 ± 0.3 | 33 ± 0.1 | 41 ± 0.5 |
| cryptolepine HLC (**2**) | 41 ± 0.6 | 62 ± 0.1 | 52 ± 0.7 |
| hydroxycryptolepine (**3**) | 31 ± 0.9 | 45 ± 0.1 | 59 ± 1.0 |
| quindoline (**4**) | 63 ± 1.1 | 87 ± 0.1 | 108 ± 1.4 |
| neocryptolepine (**5**) | 35 ± 0.7 | 51 ± 0.1 | 65 ± 1.3[†] |
| chloroquine | 2.3 ± 1.2[‡] | 72 ± 0.1 | 68 ± 0.1[†] |
| quinine | 17.6 ± 0.1 | 35.2 ± 2.0 | 102 ± 1.1 |
| mefloquine | 6.3 ± 0.1 | 4.7 ± 1.0 | 5.1 ± 0.1 |
| artemisinin | 2.5 ± 0.1[‡,§] | 3.3 ± 0.1 | 2.7 ± 0.1[§] |

[a] Values are expressed as mean ± S.D. Values within the same row or the same column are significantly different unless indicated otherwise (values bearing the same superscript are not significantly different ($p = 0.05$)).

Cryptolepine (**1**), 11-hydroxycryptolepine (**3**), quindoline (**4**), and neocryptolepine (**5**) were isolated by column chromatography combined with preparative TLC from the total alkaloid extract, while cryptolepine hydrochloride (**2**) was isolated by column chromatography from the 80% EtOH extract. All isolated alkaloids were identified as reported previously.[3,4] Compounds **1**, **3**, and **4** were also isolated from the root of *C. sanguinolenta* collected in Guinea Bissau.[5] Cryptolepine (**1**) was also obtained as its hydrochloride from some *Sida* species.[6]

The effects of isolated alkaloids as inhibitors on the incorporation of [$^3$H]hypoxanthine into *P. falciparum* strains in vitro were investigated under the same conditions as the extracts. The results, recorded in

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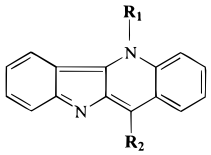
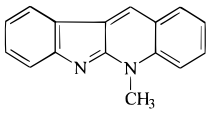


| | $R_1$ | $R_2$ |
|---|---|---|
| **1** | $CH_3$ | H |
| **2**[a] | $CH_3$ | H |
| **3** | $CH_3$ | OH |
| **4** | H | H |

[a]Hydrochloride



**5**

Table 1, indicated that all compounds were to differing degrees capable of inhibiting the growth of *P. falciparum* strains. The highest activity was obtained with cryptolepine (**1**), the major alkaloid, against D-6, K-1, and W-2 strains, with $IC_{50}$ values of 27 ± 0.3, 33 ± 0.1, and 41 ± 0.5 ng/mL, respectively. It is less active than chloroquine against *P. falciparum* D-6 (chloroquine-sensitive) but more active against *P. falciparum* K-1 and W-2, both chloroquine-resistant strains. This observation is in good agreement with Noamesi *et al.*[7] and Kirby *et al.*[8] who have reported the antiplasmodial activity of compound **1** only against the *P. falciparum* K-1 strain. These authors also demonstrated the probable interaction between cryptolepine and DNA, suggesting that this might be the final target of the alkaloid.

Cryptolepine hydrochloride (**2**) and 11-hydroxycryptolepine (**3**) exhibited a more pronounced antiplasmodial activity than chloroquine when tested against the K-1 and W-2 strains (see Table 1). Against the W-2 strain, compound **2** exhibited a significantly higher activity ($IC_{50}$, 52 ± 0.7 ng/mL) than **3** ($IC_{50}$, 59 ± 1.0 ng/mL), while against the K-1 strain, this finding was reversed. The antiplasmodial activity of neocryptolepine (**5**), a cryptolepine isomer ($IC_{50}$, 65 ± 1.3 ng/mL) was higher than that of chloroquine against the K-1 strain, but against the W-2 strain, no significant difference could be observed. Interestingly, when the antiplasmodial activity of **2** was compared to that of **5**, it was observed that cryptolepine hydrochloride (**2**) exhibited a significantly higher activity ($IC_{50}$, 52 ± 0.7 ng/mL) against the W-2 strain than did **5** ($IC_{50}$, 65 ± 1.3 ng/mL), while against the K-1 strain, this was reversed (see Table 1). The remaining alkaloid, quindoline (**4**), also exhibited antiplasmodial activity but was less potent than the other *C. sanguinolenta* alkaloids and chloroquine against all selected *P. falciparum* strains. Its $IC_{50}$ values varied from 63 to 108 ng/mL.

The in vitro antiplasmodial activity of *C. sanguinolenta* alkaloids was also compared to that of three known antimalarial drugs tested under the same conditions. The results in Table 1 indicate that the *P. falciparum* W-2 strain is resistant to quinine as previously reported by other authors.[9−13] Thus, this antimalarial drug appears to be significantly less active than cryptolepine (**1**) and its hydrochloride (**2**), 11-hydroxycryptolepine (**3**), and neocryptolepine (**5**) against the *P. falciparum* W-2 chloroquine-resistant strain. Against the K-1 strain, cryptolepine (**1**) showed a higher activity than quinine, whereas the activity of this antimlarial drug was more pronounced than that of compounds **2**, **3**, **4**, or **5**. On the other hand, artemisinin and mefloquine are more active than the test samples against D-6 (chloroquine-sensitive) and K-1 and W-2 (chloroquine-resistant) strains (see Table 1).

**Table 2.** In Vivo Antiplasmodial Activity of Extracts and Alkaloids from *Cryptolepis sanguinolenta* Root Bark[a]

| | average % parasitemia | |
|---|---|---|
| extracts/compd | *P. berghei yoelii* | *P. berghei berghei* |
| F1 | 19.4 ± 4.5* | 39.8 ±2.3[†] |
| F2 | 11.9 ± 2.3* | 18.9 ± 1.7* |
| cryptolepine (**1**) | 13.7 ± 4.3[‡] | 45.4 ± 2.2[‡] |
| cryptolepine HCl (**2**) | 10.8 ± 3.7[§] | 15.6 ± 1.7[§] |
| hydroxycryptolepine (**3**) | ND | 43.2 ± 3.5[‡] |
| quindoline (**4**) | 42.1 ± 2.1* | 48.2 ± 3.4[‡] |
| neocryptolepine (**5**) | ND | ND |
| 2.5% Tween 80 (negative control) | 34.6 ± 4.5 | 32.7 ± 3.4 |
| chloroquine (positive control) | 1.65 ± 0.05 | 2.2 ± 1.5 |

[a] Values are expressed as mean ± SD. Not significantly different from negative control (*$p = 0.01$, [†,‡]$p = 0.05$, [§]$p = 0.001$). ND: no determined.

Results of an in vivo test in Table 2 indicate that at a daily dose of 100 mg/kg of body weight, extracts F1 and F2 showed a significant chemosuppression of parasitemia against mice infected with *P. berghei yoelii*; however, F1 did not show a significant chemosuppressive effect against *P. berghei berghei* in contrast to F2, which was active in both test systems. In mice infected with *P. berghei yoelii*, at a daily dose of 50 mg/kg of body weight, compound **4** was not active, but cryptolepine (**1**) and its hydrochloride (**2**) exhibited a significant chemosuppressive effect. In mice infected with *P. berghei berghei*, compounds **1**, **3**, and **4** increased the parasitemia, and thus were considered inactive, while cryptolepine hydrochloride (**2**) showed a significant chemosuppression of parasitemia. Chloroquine, used as a positive control, was more active in vivo against both *P. berghei yoelii* and *P. berghei berghei*. In general, no correlation between the in vitro and the in vivo antiplasmodial activity of test samples could be deduced.

Although the present work confirms the in vitro activity of cryptolepine (**1**) on more *P. falciparum* strains than described before, at first view it also confirms its inactivity in vivo in mice infected with *P. berghei berghei*.,[8] but this is not consistent with the results of Wright *et al.*[5] However, it was active in vivo in mice infected with *P. berghei yoelii*, and when tested as its hydrochloride (**2**), in vivo activity in mice was found against *P. berghei yoelii* as well as *P. berghei berghei*.

The in vitro and in vivo antiplasmodial activity of other alkaloids from *Cryptolepis sanguinolenta* are reported in the present work for the first time. Although cryptolepine or its hydrochloride, the major alkaloid from *C. sanguinolenta*, is less active than known antimalarials such as mefloquine or artemisinin in vitro or chloroquine in vivo, the data reported here provide some rational evidence for the use of extracts of this plant for the treatment of malaria in traditional medicine of some African countries. Although cryptol-

epine or related alkaloids as such will probably never be used as antimalarial drugs in humans, they may provide useful lead structures for the development of a new class of synthetic antimalarials.

## Experimental Section

**General Experimental Procedures.** Chromatographic methods used for the isolation, purification, and spectroscopic techniques used for the structure elucidation of these alkaloids are detailed by Cimanga *et al.*[3,4]

**Plant Material.** The roots of *C. sanguinolenta* were collected in Kinshasa, Zaire in October 1989. The plant was identified by Mr. M. Breyne of the Institut National d'Etudes et de Recherches en Agronomie (INERA) of the University of Kinshasa, where a voucher specimen (INERA 892174) has been deposited.

**Preparation of Crude Extracts.** The dried root bark (20 g) of *C. sanguinolenta* was exhaustively macerated with distilled $H_2O$ and filtered, and the filtrate was evaporated to dryness under reduced pressure to yield a residue of 1.573 g (ca. 7.86%) (denoted as F1). Another 20-g batch of plant material was macerated and percolated exhaustively with 80% EtOH and treated in the same manner as F1 to give 2.203 g (ca. 11.01%) of dried residue (denoted as F2). In addition, 50 g of plant material was treated as described for F2, and the residue was then dissolved in 2 × 75 mL of 5% HOAc and filtered. The filtrate was made alkaline with $NH_4$-OH (pH 9−10) and extracted exhaustively with $CHCl_3$, with the $CHCl_3$ layer evaporated under reduced presure to afford 1.045 g (ca. 2.09%) of a purple extract (total alkaloid extract) denoted as F3.

**Extraction and Purification of Alkaloids.** The alkaloids from the root bark of *C. sanguinolenta* were isolated and identified as described by Cimanga *et al.*[3,4]

**In Vitro Antiplasmodial Activity**. *Plasmodium falciparum* cultures: Continuous in vitro cultures of asexual erythrocytic stages of three *P. falciparum* strains D-6 (chloroquine-sensitive) from Sierra Leone, K-1 and W-2 (chloroquine-resistant) from Thailand and Indochina, respectively, were maintained following the procedure described by Trager and Jensen[14] at 37 °C under the following conditions: atmosphere of 5% $CO_2$, 5% $O_2$, and 90% $N_2$ for D-6 and W-2 strains or 4% $CO_2$, 3% $O_2$, and 92% $N_2$ for K-1 strain. Human red blood cells were used as the host cells. *P. falciparum* D-6 and W-2 strains were inoculated into type $A^+$ human erythrocytes with a hematocrit of 6% in RPMI 1640 culture medium (Gibco) supplemented with 32 mM $NaHCO_3$ and 25 mM HEPES (*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid, Sigma Chemical Co.).[15] The K-1 strain was cultured in human $A^+$ erythrocytes suspended in RPMI 1640 supplemented with D-glucose and 10% human serum.[16]

In vitro assay of antimalarial activity: 1 mg of each test sample (extracts or pure isolated compounds) was dissolved in a minimal amount of EtOH and diluted with culture medium to give stock solutions of 100 μg/mL. Chloroquine diphosphate (Sigma) was used as an antimalarial reference, and each test sample was applied in a series of 12 fourfold dilutions in concentrations between 5 and 0.0012 μg/mL and were tested in duplicate. In vitro testing of samples was performed as described by Desjardins *et al.*[17] A suspension of 200 μL of *P. falciparum*-infected red blood cells with final parasitemia of 1% and hematocrit of 2.5% was incubated for 24 h in microtiter plates in hypoxanthine-free medium in the presence of 25 μL of test sample. Two series of controls were performed, one with infected red blood cells in the absence of sample and the other with uninfected red blood cells, again with no sample. All microtiter plates were incubated for 24 h under the same conditions mentioned before. After this incubation time, 25 μL of [$^3$H]hypoxanthine (0.5 μCi/well for D-6 and W-2 or 0.05 μCi/well for K-1) was added. The microtiter plates were reincubated for 18 h. Next, the contents of each microtiter plate were harvested into a fiber filter. The filter papers were dried for 2 h at 52 ° C. The radioactivity was measured with a Beckman LS 5801 liquid scintillation counter. The results were expressed as percentage of growth inhibition. The sigmoid dose-response was used to derive $IC_{50}$ values as the mean of two experiments. Controls without test samples defined 100% incorporation.

**In Vivo Antimalarial Activity**. The crude extracts and alkaloids from *C. sanguinolenta* were assessed for in vivo activity in a four-day suppressive test against *P. berghei yoelii* N67 and *P. berghei berghei* infections in mice.[18] Twenty-four mice (Swiss albino, mean body wt 25 g) were inoculated ($1 \times 10^7$ erythrocytes parasitized with *P. berghei yoelii*) intraperitoneally. The volume of inoculum was 0.2 mL. All test mice were infected on day 0 and were divided into 8 groups of 3 mice each. Extracts and alkaloids were dissolved in 2.5% Tween 80 and diluted with $H_2O$ to have concentrations of 100 mg/kg and 50 mg/kg of body weight for extracts and alkaloids, respectively. The volume administered orally was 0.2 mL. Six groups of mice were immediately treated orally and were given a single daily dose of test samples, while one group was given chloroquine (5 mg/kg body weight) (positive control) and the last group was given 2.5% Tween 80 (negative control) for 4 days. Another 24 mice were inoculated ($1 \times 10^7$ erythrocytes parasitized with *P. berghei berghei*) and were treated in the same manner. A thin blood film stained with Giemsa was prepared on the fifth day for each mouse, and the parasitemia was determined microscopically.

The Student's *t*-test was used to test the significance of differences between results obtained for different samples and between results for samples and controls. Statistical significance was set at $p = 0.05$.

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