Nigerian Journal of Pharmaceutical Research
Vol. 3 no. 1, pp. 42-48 (September 2004)

INDOLOQUINAZOLINE ALKALOIDS FROM *ARALIOPSIS TABOUENSIS*

C. O. Ezugwu[1*], B. Erdal[1], D. Chuck[1], I. A. Khan[1,2], C. O. Okunji[3,4], B. M. Schuster[4] and M. M. Iwu[3,4]

[1]*National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences,*
[2]*Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, USA,*
[3]*International Centre for Ethnomedicine and Drug Development, Nsukka Nigeria; and Bioresources Development & Conservation Programme 11303 Amherst Avenue Suite # 2, Silver Spring, MD 20902 USA*
[4]*Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington DC, 20307-5100, USA*

Abstract
Three new indoloquinazolidine-type alkaloids, 2-methoxy-8-13-dihydro-7H-indolo[pyrido[2,1-b]quinazolin-5-one **(1)**, 2-methoxy-13-methyl-8-13-dihydro-7H-indolo[2',3',3,4]pyrido[2,1-b]quinazolin-5-one **(2)**, and 2-methoxy-14-methyl-7,8,13,14-tetrahydroindolo[2',3',4]pyrido[2,l-b]quinazolin-5-one **(3)** were isolated from Araliopsis tabouensis, together with three known compounds. The structures of the new compounds were determined primarily from 1D- and 2D-NMR spectroscopy. The antimalarial activities of compounds **1-5** were evaluated against Plasmodium falciparum D6 and W2 clones. The IC_{50} values in antimalarial bioassay for compounds **2-5** varied from 1.8 to 4.7 μg/ml.

INTRODUCTION

Araliopsis tabouensis Aubrev. Et Pedllegr. (Rutaceae) is a large evergreen tree which grows in the tropical forest of West and Central Africa. Its medicinal use is for the treatment of sexually transmitted diseases. An infusion of the exceedingly bitter tasting bark is drunk as a cure for gonorrhea in the Ivory Coast [1]. In the previous chemical investigations of *A. tabouensis* large quantities of the protolimonoid triterpene flindissol, quinoline and indoloquinazoline type alkaloids were isolated and identified [2-4].

As part of our ongoing biological evaluation of West African medicinal plants, we undertook a bioactivity-guided phytochemical investigation of the stem bark of *A. tabouensis*, which resulted in the isolation of indoloquinazoline and quinoline alkaloids. Their structures were assigned by spectroscopic methods [IR, HRESIFTMS, 1D-(^{1}H and ^{13}C), and 2D-NMR (G-DQF-COSY, G-HMBC, and ^{15}N-^{1}H G-HMBC]. The present study describes the structure

* Corresponding author. (Current Address: Department of Pharmacognosy, University of Nigeria, Nsukka)
E-mail address: oechristo@yahoo.co.uk
ISSN 0189-8434

elucidation of the new indoloquinazoline alkaloids (**1-3**).

MATERIALS AND METHODS

1. General: The 1D- and 2D-NMR spectra were obtained on a Bruker® Avance DRX 500 FT spectrometer operating at 500 (^{1}H) and 125 (^{13}C) MHz. The chemical shift values are reported as parts per million (ppm) relative to tetramethylsilane (TMS) for ^{1}H- and ^{13}C- and the coupling constants are in Hz (in parentheses). For the ^{13}C-NMR spectra, multiplicities were determined by a DEPT experiment. 15N chemical shifts are relative to liquid ammonia by calibrating nitromethane to δ 380.2 HRESIFTMS (High Resolution Electrospray Ionization Fourier Transformation Mass Spectrometry) were obtained using a Bruker Bio apex FT-MS in ESI (+) mode.

2. Chromatographic Condition: TLC precoated Si 250F plates (Baker); developing system; $CHCL_3$-MeOH mixtures (90:10, 85:15), $CHCL_3$-MeOH-H_2O mixtures (80:20:1, 80:20:2, 70:30:3); visualization: Dragendoff's reagent: Column chromatography: silica gel 230-400 mesh, RP (C-18, 40 mμ) Merck), Sephadex (LH-20).

3. Plant Material: The plant material was collected from Owi, Cross River State, Nigeria in December 2001 and identified by Mr. Ozioko Alfred, a taxonomist at the Bioresources Development and Conservation Programme (BDCP) at Nsukka. Voucher specimens are deposited at the BDCP herbarium.

4. Isolation and Purification: The alkaloid extract (8.0 g) was chromatographed on silica and eluted with increasing amounts of MeOH in $CHCl_3$. Identical fractions were pooled into 17 major fractiosn (I-XVII) according to their TLC profiles. Fraction VII (314 mg) was purified by gel filtration (Sephadex LH-20) in methanol, followed by centrifugal chromatography (chromatotron system, Model 8924, 4 mm plate, flow rate: 8-10 mL/min.) using chloroform-methanol mixtures to obtain 200 mg of **3**. Fractions XIII and XIV (59.7 mg and 49.8 mg, respectively) precipitated to give a total of 109.5 mg of **1** (EC-AT-02). Fraction VIII (65.6 mg) was chromatographed on chromatotron using chloroform-methanol mixtures to obtain 15.7 mg of **2** (EC-AT-06). Further chromatographic studies (SiO_2, Sephadex LH-20, chromatotron) resulted in the isolation of known compounds 4-7 (yield: 24.6, 44.5, 13.2, 12.4 mg, respectively).

5. Antimalarial assay: The *in* vitro antimalarial activity was determined against two strains of *Plasmodium* falciparum, D6 (chloroquine sensitive) and W2 (chloroquine resistant). The assay is based on the determination of parasite lactate dehydrogenase activity (LDH) using Malstat reagent [9]. The two clones are subcultered daily with fresh medium and blood cells, gassed with a mixture of 90 % N_2, 5 % O_2, and 5 % CO_2 and incubated at 37 ^oC. On the day of assay, a suspension of infected red blood cells (2 % parasitemia and 2 % hematocrit) is prepared using type A human red blood cells (Interstate Blood Bank, Memphis, TN) in RPMI 1640 medium supplemented with 10 % human serum (Interstate Blood Bank, Memphis, TN) and amikacin (60 mg/ml, Sigma, St. Louis, MO). To a 96 well flat-bottomed microplate, 200 μl of the cell suspension is added along with 10 μl of the samples, which have been diluted in the medium, in duplicate. The plate is placed into a modular incubation chamber (Billups-Rothenberg, CA) and flushed with the gas mixture. The chamber containing the plates is then placed in a 37 ^oC incubator for 48 hours. After incubation the cultures are mixed and 20 μl from each well is transferred to another microplate containing the Malstat™ reagent, and this plate is incubated at room temperature for 30 minutes. 20 μl of a 1:1 mixture of Nitro Blue Tetrazolium/Piperazine N,N'-bis(2-ethane sulfonic acid) (Sigma, St.

Louis, MO) is added, and the plate is incubated in the dark for one hour. The reaction is then stopped by the addition of 100 µl of a 5 % acetic acid solution to each well. The plate is read at 650 nm on an EL-340 Biokinetic Reader (Bio-Tek Instruments, Vermont). IC_{50} is calculated from dose curves of growth inhibition. Chloroquine and artemisin are included as control drugs in each assay.

RESULTS AND DISCUSSION

Bioassay-guided fractionation of *A. tabouensis* resulted in the isolation of three new indoloquinazoline alkaloids (**1-3**), as well as three known compounds, veprisine (**4**). N-methyl preskimmianine (**5**), and scopoletin (**6**). The known compounds were identified comparison of their spectral data with the reported values [4, 5].

Compound **1** was isolated as a yellow amorphous powder. The molecular formula of 1 was determined as $C_{19}H_{15}N_3O_2$ by HRESIFTMS, which exhibited ions at *m/z* 318.1247 $[M+H]^+$, 340.1064 $[M+Na]^+$ and 635.2321 $[2M+H]^+$ (positive mode).

The ^{1}H-NMr spectrum of **1** exhibited a deshielded proton at δ 13.1 ppm (s, N_{13}-H), an O-methyl at δ 3.77 (s, 3H), and two symmetrical triplets at δ 3.07 (t, *J*=6.3 Hz) and 4.55 (t, *J*=6.3 Hz). The chemical shifts of the latter protons (δ 3.07 and 4.55) are typical of the C-7 and C-8 methylene protons of quinazolinecarboline (indoloquinazoline) type alkaloids. This assumption was supported by the ^{13}C-NMR spectral data, which indicated characteristic C-7 and C-8 methylene carbon signals at δ 41.3 and 19.9 respectively, based on the G-HMQC connectivities. In addition, the ^{1}H-NMR spectrum showed seven aromatic protons: an *ortho*-disubstituted (δ_{H-10} 7.27, t, *J*=7.5 Hz; δ_{H-11} 7.44, t, *J*=7.6 Hz; δ_{H-9} 7.74, d, *J*-7.8 Hz; δ_{H-12} 7.80, d, *J*=8.0 Hz) and one trisubstituted-aromatic ring (δ_{H-3} 7.09, d, *J*-8.8 Hz; δ_{H-1} 7.15 br s; δ_{H-4} 8.44, d, *J*=8.8 Hz). The substitution pattern, and the ^{1}H- and ^{13}C-NMR chemical shift values of the *ortho*-disubstituted aromatic ring (δ_{C-9} 120.7, δ_{C-10} 120.6, δ_{C-11} 125.5 and δ_{C-12} 113.1; ring A), and the signals attributed to C- and D rings (above mentioned methylene signals, and δ_{C-8a} 118.5, δ_{C-13a} 128.6, δ_{C-14a} 150.7, δ_{C-4a} 115.6 and δ_{C-5} 161.3, each quaternary carbon) were in clear agreement with those reported for rutaecarpine, except for the differences associated with the presence of a methoxyl group (E-ring).

The combined use of G-DQF-COSY, G-HMQC and G-HMBC spectra of **1**, permitted the complete assignment of the indoloquinazoline skeleton and its substitution pattern. Thus, the deshielded ^{1}H-NMR signal at δ 13.11 showed correlations with two quaternary carbons at δ 140.0 and 128.6, which were attributed to C-12a and C-13a, respectively. The aromatic proton observed at δ 8.44 (H-4) indicated that it was adjacent to an electron-withdrawing substituent. This assumption was supported by long-range correlation in the G-HMBC spectrum between this proton, attributed to H-4, and a carbon signal at δ 161.3 (C-5), assigned to the amide carbonyl. The H-4 proton also exhibited HMBC connectivity with another downfield shifted carbon at δ 164.8 (C-2) indicating an electron-donating group, either a hydroxyl or a methoxyl group. The latter showed correlations with the *O*-methyl protons (δ 3.77 in the G-HMBC spectrum, hence allowing it to be assigned unambiguously to C-2, and locating the methoxyl group to C-2. Moreover, the position of the methoxyl group were confirmed based on the ^{1}H-^{15}N-NMR HMBC correlations between H-1 (δ 7.15, br s) and the nitrogen signal observed at δ 223.1 (N-14), which ruled out the possibility of C-3 methoxyl substitution.

Based on these results, the structure of **1**, a new natural product, was established as 2-methoxyrutaecarpine {2-methoxy-8,13-

dihydro-7*H*-indolo[2',3':3,4]pyrido[2,1-*b*]quinazolin-5-one}.

The HRESIFTMS of **2** displayed a molecular ion at *m/z* 332.1405 $[M+H]^+$ ($C_{20}H_{17}N_3O_2$), which was 14 mass units higher than that of **1**, confirming the presence of an extra methyl group. The signals arising from the indoloquinazoline skeleton of **2** and **1** were superimposible, with the exception of an additional signal at δ 4.45 (s, 3H) in the ^{1}H-NMR spectrum, implying the presence of an *N*-methyl group. The assignment of ^{1}H and ^{13}C signals of **2** was secured by G-DQF-COSY, G-HMQC, and G-HMBC spectra. The location of the extra methyl group was confirmed by a ^{1}H-^{15}N-NMR HMBC experiment, which showed a correlation between the *N*-methyl protons resonating at δ 4.45 and nitrogen signal at δ 131.0, attributed to N-13.

Based on these observations, the structure of **2** was elucidated as 2-methoxy-13-methylrutaecarpine {2-methoxy-13-methyl-8,13-dihydro-7*H*-indolo[2',3':3,4]pyrido[2,1-*b*]quinazolin-5-one}.

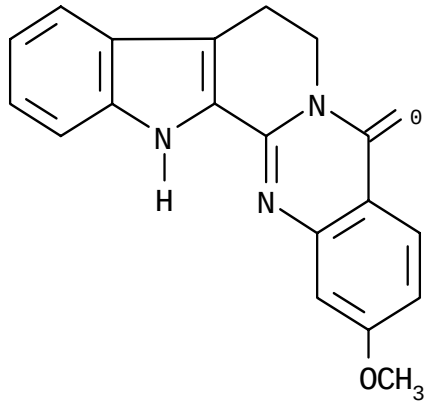
Compound **3** was isolated as a yellow amorphous solid. The HRESIFTMS of **3** displayed a molecular ion at *m/z* 332.1313 $[M]^+$, supporting a molecular formula of $C_{20}H_{18}N_3O_2$. Inspection of the ^{1}H-NMR spectrum of **3** displayed the typical spin patterns of compound **1** and **2**: seven aromatic proton signals, of which two appeared as triplets (t) at δ 7.28 (*J*=7.5 Hz), and 7.46 (*J*=7.5 Hz) attributed to H-10 and H-11, four were doublets (d) at δ 7.73 (*J*=8.5 Hz), 7.77 (*J*=8.5 Hz), 7.79 (*J*=9.0 Hz), and 6.22 (*J*=1.0 Hz) assigned to H-12, H-9, H-4, and H-1, and one doublet of doublets (dd) at δ 6.31 (*J*=9.5, 1.0 Hz) attributed to H-3, a methoxy signal at δ 3.66 (s, 3H), the symmetrical triplets at δ 3.38 (2H, *J*=6.3 Hz) and 4.58 (2H, *J*=6.3 Hz) correlating with carbons at δ 47.2 (t), and 20.3 (t) in the G-HMQC spectrum, indicating the presence of C-7 and C-8 methylenes. Three nitrogen signals at δ 149.0, 128.9 and 63.2 correlating with proton signals at δ 3.38 (H_2-8), 13.3 (N_{13}-H) and 2.71 (N_{14}-CH_3), respectively, were observed in the ^{15}N-^{1}H NMR HMBC spectrum. Detailed examination of ID and 2D NMR spectra of **3** indicated that **3** differs from **2** and **1** in the position of the double bond in ring C/D (N-6=C-14a for **3**; N-14=C-14a for **2**), implying a quaternary nitrogen, and the position of N-methyl group (N-14 for **3**; N-13 for **2**).

In regard to the rearrangement of the C- and D rings, five factors were taken into consideration: (i) the nitrogen signal attributed to N-6 was observed at δ149.0, ~15.0 ppm upfield shifted compared to the N-6 signal of compound **1** (δ 164.0 ppm); (ii) The upfield shift of H-1 (δ 6.22, *J*=1.0 Hz for **3**; δ 7.15, br s for **1**), which is not deshielded due to the absence of the double bond between N-14 and C-6a. (iii) The downfield shift of C-5 and C-14 (C-5; δ 175.1 for **3**; δ 161.3 for **1**, ~14 ppm; C-14 δ 161.3 for **3**; δ 146.6 for **1**, ~15 ppm) because of the positive charge on N-6. (iv) ^{15}N-^{1}H-NMR, HMBC correlations between N-6 (δ 149.0) and methylene protons of C-7 and C-8 (δ 3.38, 4.58), and between N-14 (δ 63.2) and methyl signal at δ 2.71 and aromatic proton signal at δ 6.22 (H-1). (v) The HRESIFTMS spectra of **1** and **2** provided $[M+H]^+$ and $[M+Na]^+$ ions, while the HRESIFTMS of **3** only gave a molecular ion $[M]^+$ due to the positive charge on the molecule.

On the basis of this evidence, the structure of **3** was established as 2-methoxy-14-methyl-7,8,13,14-tetrahydro-indolo[2',3':3,4]pyrido[2,1-*b*]quinazolin-5-one.

Compounds **2-5** were active against *Plasmodium falciparum* D6 and W2 clones (Table 1). Although compounds **1-3** were from the same chemical class with minor differences, only compounds **2** and **3** exhibited significant antimalarial activity,

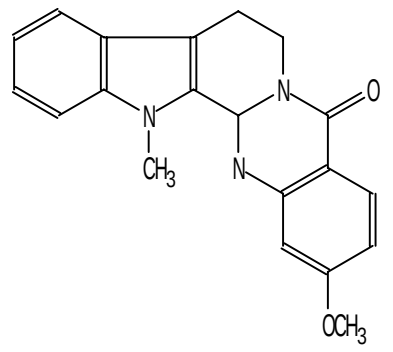
while compound **1** unexpectedly lacked activity as shown in Table 1.



Compound 1

2-Methoxyrutaecarpine (EC-AT-02) $C_{19}H_{15}N_3O_2$, yellow amorphous powder, M.P. 253°, violet fluorescent spot, Elemental analysis (%) C. 71.91; H, 4.76; N, 13.24; O, 10.08.

^{1}H NMR (500 MHz, in C_5D_5N) δ 13.11 (1H, s, N_{13}-H), 8.44 (1H, d, J=8.8, H-4), 7.80 (1H, d, J=8.0 Hz, H-12), 7.74 (1H, d, J=7.8 Hz, H-9), 7.44 (1H, t, J=7.6 Hz, H-11); 7.27 (1H, t, J=7.5 Hz, H-10), 7.15 (1H, brs, H-1), 7.09 (1H, d, J=8.8 Hz, H-3), 4.55 (2H, t, J=6.8 Hz, H_2-7), 3.77 (3H, s, Ome), 3.07 (2H, t, J=6.8 Hz, H_2-8); ^{13}C NMR (125 MHz, in C_5D_5N): 164.8 (s, C-2), 161.3 (s, C-5), 150.7 (s, C-1a), 146.6 (s, C-14a), 140.0 (s, C-12a), 129.1 (d, C-4), 128.6 (s, C-13a), 126.3 (s, C-9a), 125.5 (d, C-11), 120.7 (d, C-10), 118.5 (s, C-8a), 115.6 (s, C-4a), 115.7 (d, C-3), 113.1 (d, C-12), 108.5 (d, C-1), 55.7 (q, Ome), 41.3 (t, C-7), and 19.9 (t, C-8); HRESIFTMS at m/z $[M+H]^+$ calcd. For $C_{19}H_{15}N_3O_2$, 318.1242; found 318.1247, $[M+Na]^+$ 340.1064 and $[2M+H]^+$ 635.2321.

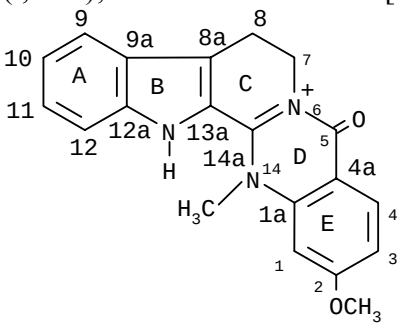


Compound 2

2-Methoxy-13-methylrutaecarpine (EC-AT-06) $C_{20}H_{17}N_3O_2$, Yellow amorphous powder; M.P. 164, brilliant blue fluorescent spot, Elemental analysis (%) C, 72.49; H, 5.17; N, 12.68; O, 9.66.

^{1}H NMR (500 MHz, in $CDCl_{3S}$) δ 8.02 (1H,d, J=9.0 Hz, H-4), 7.84 (1H,d,J=8.2 Hz, H-12), 7.81 (1H,d,J=2.6 Hz, H-1), 7.69 (1H,d, J=8.4 Hz, H-9); 7.67 (1H,dd,J=2.6, 9.0 Hz, H-3), 7.53 (1H,t,J=7.5 Hz, H-11), 7.30 (1H,t,J=7.5 Hz, H-10), 4.58 (2H,t,J=6.7 Hz, H_2-7), 4.45 (3H,s, N-Me), 4.00 (3H, s, Ome), 3.39 (2H,t,J=6.7 Hz, H_2-8); ^{13}C NMR (125 MHz, in $CDCl_3$; 161.8 (s, C-2), 160.1 (s, C-5), 150.6 (s, C-1a), 144.1 (s, C-14a), 135.7 (s, C-12a), 132.3 (s, C-13a), 130.9 (d, C-4), 126.8 (s, C-9a), 125.8 (d, C-11), 123.9 (d, C-10), 123.0 (d, C-9), 122.3 (s, C-8a), 121.9

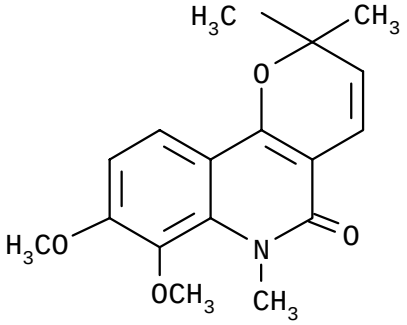
(d, C-12), 121.7 (s, C-4a), 115.0 (d, C-3), 111.0 (d, C-1), 57.4 (q, Ome), 42.1 (t, C-7), and 20.7 (t, C-8); HRESIFTMS at m/z $[M+H]^+$ calcd. For $C_{20}H_{17}N_3O_2$, 332.1400; found 332.1405.



Compound 3

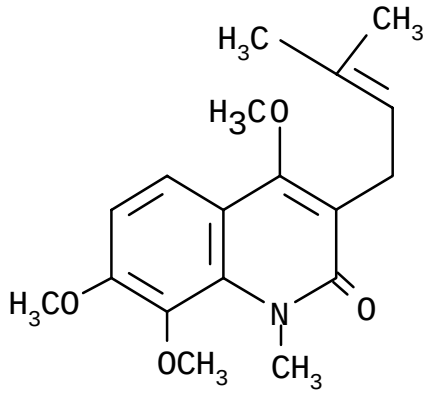
2-methoxy-14-methyl-7,8,13,14-tetrahydroindolo[2',3':3,4]pyrido[2,1-b]quinazolin-5-one, $C_{20}H_{18}N_3O_2$, Yellow amorphous powder, M.P. 208-209 ^oC, elemental analysis (%): C, 72.27; H, 5.46; N, 12.64; O, 9.63.

^1H NMr (500 MHz, in C_5D_4N) δ 13,30 (1H, s, N_{13}-H), 7.79 (1H, d, J=8.8, H-4), 7.77 (1H,d,J=8.0 Hz, H-9), 7.73 (1H, d, J=7.5 Hz, H-12), 7.46 (1H,t,J=7.5 Hz, H-11); 7.28(1H,t,J=7.5 Hz, H-10), 6.31 (1H,dd,J=9.0 and 1.0 Hz, H-3), 6.22 (1H,d, J=1.0 Hz, H-1), 4.18 (2H,t, J=6.3 Hz, H_2-7), 3.66 (3H,s, Ome), 3.38 (2H,t,J=6.3 Hz, H_2-8); ^{13}C NMR (125 MHz, in C_5D_5N): 175.1 (s, C-5), 164.6 (s. C-2), 161.3 (s, C-14a), 153.3 (s, C-1a), 138.4 (s, C-12a), 135.2 (d, C-4), 126.9 (s, C-13a), 124.9 (d, C-11), 124.7 (s, C-9a), 121.3 (s, C-8a), 120.3 (d, C-9), 119.6 (d, C-10), 112.2 (d, C-12), 109.1 (s, C-4a), 101.8 (d, C-3), 94.0 (d, C-1), 55.7 (q, Ome), 47.2 (t, C-7), and 20.3 (t, C-8); HRESIFTMS at m/z$[M]^+$ calcd. For $C_{20}H_{18}N_3O_2$, 332.1399; found 332.1313.



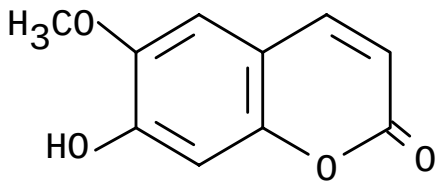
Compound 4

7,8-dimethoxy-N-methylflindersine (Veprisine)



Compound 5

7,8-dimethoxy-3-(3-methylbut-2-enyl)-2-quinoline (N-methylpreskimmianine)



Compound 6

6-methoxy-7-hydroxycoumarin (Scopoletin), $C_{10}H_8O_4$;

Table 1: Antiprotozoal activities of compounds **1-5** (IC_{50}, μg/ml)

Compounds	*P. falciparum* (D6 Clone)	P. falciparum (W2 Clone)	Cytotoxicity (Vero)
1	NA	NA	NC
2	1.8	>4.7	NC
3	3.3	>4.7	NC
4	2.0	>4.7	NC
5	2.1	1.8	NC
Artemisinin	<0.026	<0.026	-

NA = Not active; NC = No cytotoxicity

ACKNOWLEDGEMENT

This work was supported in part by the United States Department of agriculture, ARS Specific Cooperative Research Agreement No. 58-6408-2-0009, and National Institutes of Health (NIH) through ICBG grant No. TW03004. We thank Dr. S. I. Khan, Dr. M. Jacob, M. Wright and J. Trott for performing antimalarial test.

REFERENCES

1. F. R. Irvine, 'Woody Plants of Ghana', Oxford University Press, **1961**, 491.
2. F. Fish, I. A. Meshal, P. G. Waterman, *Planta Medica* **1976**, 29, 310.
3. P. G. Waterman, Biochemical Systematics and Ecology **1973**, 1, 153.
4. B. T. Ngadjui, J. F., Ayafor, B. L., Sondengam, M., Koch, F., Tillequin, J. D., Connoly. *Phytochemistry* **1988**, 27, 2979.
5. J. F. Ayafor, B. L. Sondengam, B. T. Ngadjui, *Phytochemistry* **1988**, 21, 2733.
6. J. Bergman, S. Bergman, *J. Org. Chem.* **1985**, 50, 1246.
7. T. Kametani, T. Higa, C. V. Loc, M. Ihara, M. Koizumi, K. Fukumoto, *J. Am. Chem. Soc.* **1976**, 98, 6186.
8. M. shaman, D. M. Hindenlang, 'Carbon-13 NMR Shift Assignments of Amines and Alkaloids". Plenum Press, New York and London, **1979**, 237.
9. M. T. Makler, D. J. Hinrichs, *Am. J. Trop. Med. Hyg.* **1993**, 48, 205.