Cytotoxic and Antimalarial Constituents of the Roots of Eurycoma longifolia

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CYTOTOXIC AND ANTIMALARIAL CONSTITUENTS OF THE ROOTS OF *EURYCOMA LONGIFOLIA*[1]

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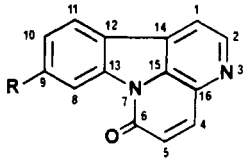
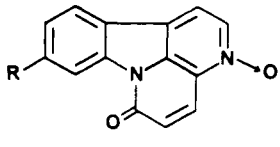
ABSTRACT.—By bioactivity-directed fractionation, five cytotoxic constituents have been characterized from the roots of *Eurycoma longifolia* collected in Kalimantan, Indonesia. Four canthin-6-one alkaloids, namely, 9-methoxycanthin-6-one [**1**], 9-methoxycanthin-6-one-*N*-oxide [**2**], 9-hydroxycanthin-6-one [**3**], and 9-hydroxycanthin-6-one-*N*-oxide [**4**], and one quassinoid, eurycomanone [**5**], were found to be cytotoxic principles. Each of these compounds was evaluated against a panel of cell lines comprising a number of human cancer cell types [breast, colon, fibrosarcoma, lung, melanoma, KB, and KB-V1 (a multi-drug resistant cell line derived from KB)] and murine lymphocytic leukemia (P-388). The canthin-6-ones **1–4** were found to be active with all cell lines tested except for the KB-V1 cell line. Eurycomanone [**5**] was inactive against murine lymphocytic leukemia (P-388) but was significantly active against the human cell lines tested. Two additional isolates, the β-carboline alkaloids β-carboline-1-propionic acid [**6**] and 7-methoxy-β-carboline-1-propionic acid [**7**], were not significantly active with these cultured cells. However, compounds **5** and **7** were found to demonstrate significant antimalarial activity as judged by studies conducted with cultured *Plasmodium falciparum* strains. The structures of the novel compounds **2–4** and **7** were established by spectral and chemical methods.

In a continuing collaborative search for naturally occurring medicinal agents, the roots of *Eurycoma longifolia* Jack (Simaroubaceae) collected from Kalimantan, Indonesia, were selected for investigation. In Indonesia and Malaysia, this plant has the local names Pasakbumi and Tongkat Ali, and the roots are used as a traditional treatment for dysentery, glandular swelling, persistent fever, and tertian malaria (2–4). Previous studies on the chemical constituents of *E. longifolia* roots have led to the isolation of several quassinoids and a canthin-6-one alkaloid (3–10). Certain C-20 quassinoids from this species with either an α,β-unsaturated keto- or a diol-type ring A were found to exhibit antimalarial activity (8,9). Extracts derived from the roots of this plant were also found to demonstrate activity when evaluated with the sarcoma 180 model (10). Recently, the cytotoxic activity of several quassinoids isolated from this plant was reported using cultured KB and P-388 cells (11).

We now report four alkaloids, namely, 9-methoxycanthin-6-one [**1**], 9-methoxycanthin-6-one-*N*-oxide [**2**], 9-hydroxycanthin-6-one [**3**], and 9-hydroxycanthin-6-



1 R=OMe
3 R=OH



2 R=OMe
4 R=OH

[1]Paper No. IV in the series, "Studies on Indonesian Medicinal Plants." For Part III, see Dai *et al.* (1).

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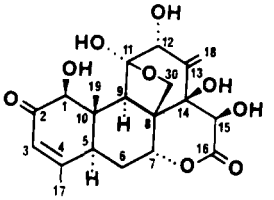
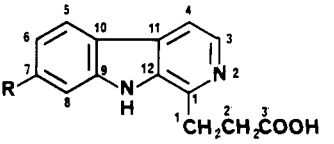
one-*N*-oxide [**4**], and a quassinoid, eurycomanone [**5**], as cytotoxic constituents of *E. longifolia* roots. In addition to these active constituents, we have also isolated two β-carboline alkaloids for the first time from this plant, i.e., β-carboline-1-propionic acid [**6**] and 7-methoxy-β-carboline-1-propionic acid [**7**]. These two compounds were not cytotoxic. However, 7-methoxy-β-carboline-1-propionic acid [**7**] was found to be active when evaluated for antimalarial potential utilizing cultured *Plasmodium falciparum*, as was eurycomanone [**5**]. Four of the compounds obtained, **2**–**4** and **7**, are of novel structure.



5



6 R=H
7 R=OMe

RESULTS AND DISCUSSION

In an initial study, an MeOH-soluble extract of *E. longifolia* roots was found to be cytotoxic when tested in the in vitro P-388 murine lymphocytic leukemia assay system. As a result of subjecting this MeOH extract to Si gel cc, two of nine combined fractions were found to demonstrate significant cytotoxic activity (P-388 ED_{50} <20 μg/ml). One of these two fractions yielded compounds **1** and **2**, and the other yielded **3**–**7**.

The physical and spectral data of compound **1** matched those of 9-methoxycanthin-6-one, which has been isolated from *Simaba cuspidata* Spruce ex Engl. (12) and *Simaba multiflora* A. Juss. (13). Using ^{1}H-^{13}C HETCOR and ^{1}H-^{13}C COLOC nmr experiments, its ^{13}C-nmr chemical shifts were assigned unambiguously (Table 1). These ex-

TABLE 1. ^{13}C-nmr Assignments for Compounds **1**–**4**.

Carbon	Compound			
	1[a]	**2**[a]	**3**[b]	**4**[b]
C-1	115.59	116.63	116.01	117.54
C-2	146.02	135.84	145.98	135.50
C-4	139.92	129.97	140.03	129.86
C-5	128.56	127.83	128.04	127.13
C-6	159.61	159.11	158.94	159.87
C-8	101.41	101.62	102.98	103.18
C-9	163.20	161.68	160.50	163.83
C-10	114.26	114.72	114.00	114.42
C-11	123.47	122.54	124.65	123.59
C-12	117.03	116.77	115.57	116.64
C-13	142.02	138.80	140.53	138.00
C-14	129.24	121.73	129.88	121.80
C-15	131.16	129.82	131.66	130.24
C-16	136.04	126.25	135.03	126.76
OCH_3	56.03	56.03	—	—

[a]Recorded in $CDCl_3$.
[b]Recorded in DMSO-d_6.

periments also corroborated the placement of the methoxy group at C-9. Moreover, compound **1** was directly comparable (mmp, uv, ir, ^{1}H-nmr, ^{13}C nmr, ms, co-tlc) to an isolate obtained earlier in this laboratory from the wood of *S. multiflora*, which was assigned as 10-methoxycanthin-6-one using primarily low resolution ^{1}H-nmr spectroscopy (14). Therefore, this *S. multiflora* isolate has now been reassigned as 9-methoxycanthin-6-one.

Compound **2** exhibited a molecular formula of $C_{15}H_{10}N_2O_3$, based on its high resolution eims data. The uv spectrum was similar to those reported for canthin-6-one-*N*-oxide and 1-methoxycanthin-6-one-*N*-oxide isolated from *Ailanthus altissima* Swingle (15). No bathochromic or hypsochromic shift was observed on addition of base. The ^{1}H- and ^{13}C-nmr spectra were comparable to those measured for 9-methoxycanthin-6-one [**1**], except that the ^{1}H-nmr signal for H-4 was shifted 0.5 ppm downfield, and the signal for H-2 was shifted 0.5 ppm upfield, relative to the same resonances in compound **1**. Similarly, the ^{13}C-nmr resonance of C-2 was found at δ 135.84, about 11 ppm upfield compared to the analogous chemical shift of compound **1** (δ 146.02). The latter observation is in accordance with a similar C-2/C-6 shift apparent in the ^{13}C-nmr spectra of pyridine and pyridine-*N*-oxide (16). Evidence was thus obtained that compound **2** was the *N*-oxide of compound **1**. Unambiguous ^{1}H- and ^{13}C-nmr assignments for **2**, obtained through the application of 1D and 2D nmr techniques, are shown in Tables 2 and 1, respectively. Final proof of the structure of this compound was obtained by comparison of its physical and nmr data with those of 9-methoxycanthin-6-one-*N*-oxide [**2**] prepared from compound **1**.

9-Hydroxycanthin-6-one [**3**] was obtained in the form of small yellow needles, and its physical and spectral data were very similar to published data for 10-hydroxycanthin-6-one (8,14). Its uv spectrum showed a bathochromic shift on addition of base, thereby suggesting the presence of a phenolic functionality. ^{1}H- and ^{13}C-nmr resonances of this compound were quite comparable to those obtained for compound **1**. After measuring the ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR nmr spectra of **3** and following comparison with published unambiguous ^{1}H- and ^{13}C-nmr assignments for canthin-6-one (17), the hydroxy group was tentatively placed at C-9. In a selective INEPT experiment ($^3J_{CH} = 8$ Hz) performed with **3**, irradiation of H-2 (δ 8.76) enhanced the resonances of C-14 (δ 129.88) and C-16 (δ 135.03), while irradiation of H-11 (δ 8.17) enhanced the resonances of C-14 (δ 129.88), C-9 (δ 160.50), and C-13 (δ 140.53). The data obtained from a ^{1}H-^{13}C COLOC experiment also supported the placement of the hydroxy group at C-9. Final proof of the structure of **3** was obtained by methylation with CH_2N_2 to afford compound **1**. Compound **3** was directly comparable (mmp, uv, ir, ^{1}H nmr, ^{13}C nmr, ms, co-tlc) with 10-hydroxycanthin-6-one obtained earlier in this laboratory as an isolate from wood of *S. multiflora*; the previous assignment was based on ^{1}H-nmr measurements made without the benefit of supportive ^{13}C-nmr spectroscopy and contemporary confirmatory 1D and 2D nmr pulse sequences (14). Therefore, this *S. multiflora* isolate has been reassigned as 9-hydroxycanthin-6-one [**3**]. Also, since an earlier report of 10-hydroxycanthin-6-one in *E. longifolia* roots was based on physical and spectral data comparison with 10-hydroxycanthin-6-one from *S. multiflora* (8), it is likely that the canthin-6-one alkaloid isolate of Chan *et al.* (8) is also 9-hydroxycanthin-6-one.

9-Hydroxycanthin-6-one-*N*-oxide [**4**] was obtained as red needles. In MeOH solution, this compound exhibited an intense yellow-green fluorescence. A molecular formula of $C_{14}H_8N_2O_3$ was obtained from the hreims spectrum of **4**. The uv spectrum was very close to that of compound **2**, and on addition of base, a bathochromic shift was observed. Relative to compound **3**, the H-2 ^{1}H-nmr resonance was shifted upfield by 0.4 ppm and the H-4 resonance shifted downfield by 0.2 ppm, while the ^{13}C-nmr reso-

nances of C-2 and C-16 were shifted about 11 ppm upfield. Unambiguous nmr assignments are shown in Tables 1 and 2. Methylation of compound **4** with CH_2N_2 afforded compound **2**.

TABLE 2. ^{1}H-nmr Assignments for Compounds **2–4**.[a]

Proton	Compound		
	2[a]	**3**[b]	**4**[b]
H-1	7.70 (d, 5.5)	8.14 (d, 5.0)	8.06 (d, 5.5)
H-2	8.31 (d, 5.5)	8.76 (d, 5.0)	8.36 (d, 5.5)
H-4	8.40 (d, 10)	8.10 (d, 10)	8.30 (d, 10)
H-5	6.94 (d, 10)	6.96 (d, 10)	6.92 (d, 10)
H-8	8.20 (d, 2.5)	8.00 (d, 2.5)	7.94 (d, 2.5)
H-10	7.10 (dd, 9, 2.5)	7.00 (dd, 9, 2.5)	6.98 (dd, 9, 2.5)
H-11	7.84 (d, 9)	8.17 (d, 9)	8.04 (d, 9)
OMe	3.98 (s)	—	—

[a]Recorded in $CDCl_3$.
[b]Recorded in DMSO-d_6.

Compound **5** was isolated as white cubic crystals and identified as eurycomanone, a quassinoid already known as a constituent of *E. longifolia* roots, by comparison of its physical and spectral data with published values (3,8). The ^{13}C-nmr values (3) for this compound have been revised as a result of the interpretation of ^{1}H-^{1}H COSY, ^{1}H-^{13}C HETCOR and ^{1}H-^{13}C COLOC nmr experiments.

Compounds **6** and **7** gave uv spectra suggestive of structures based on the β-carboline skeleton (18–20). Compound **6** was identified as β-carboline-1-propionic acid by comparison of its physical and spectroscopic data with published values for this compound obtained from *A. altissima* (21). Compound **7** exhibited a molecular formula of $C_{15}H_{14}N_2O_3$ as determined by its hreims. The ^{1}H- and ^{13}C-nmr spectra of compound **7** were similar to those of compound **6**, but evidence of an additional methoxy group could be inferred from extra resonances in its ^{1}H-nmr (δ 3.88) and ^{13}C-nmr (δ 55.32) spectra. The methoxy group was assigned at position C-7 based on a ^{1}H-^{1}H NOESY experiment wherein cross peaks were found between the methoxy group and H-8, which in turn had a cross peak with N-H. The performance of a selective INEPT experiment also supported the methoxy group attachment at C-7, since irradiation ($^3J_{CH}$ = 8 Hz) of H-3 (δ 8.18) gave enhancements of C-11 (δ 127.44), while irradiation of H-5 (δ 8.05) gave enhancements of C-11 (δ 127.44), C-9 (δ 142.01), and C-7 (δ 160.12). The ^{1}H-^{13}C COLOC nmr spectrum of compound **7** also was in agreement with the placement of the methoxy group at C-7.

In previous work, several canthin-6-one alkaloids that were hydroxylated and methoxylated at the C-10 and C-11 positions have been found to exhibit significant cytotoxicity against cultured KB cells (14,17,22). We have now been able to show that such activity is also exhibited by canthin-6-one alkaloids that are oxygenated at the C-9 position (Table 3). The four canthin-6-one alkaloids **1–4** obtained in the present investigation were generally cytotoxic to all tumor cell lines represented, with the exception of the KB-V1 cell line (drug-resistant KB cells). As a general trend, the *N*-oxides **2** and **4** were somewhat less active in all of the bioassays in which they were evaluated as compared to their respective free base analogues **1** and **3**. Eurycomanone [**5**] was not active against P-388 cells but demonstrated a general cytotoxic response with all of the epithelial cell types (including KB-V1). In addition, the most intense response was observed

TABLE 3. Evaluation of the Cytotoxic Potential of Isolates **1–5**.[a]

Compound	Cell Line[b]							
	A	B	C	D	E	F	G	H
1	2.1	>20	3.5	4.5	4.0	2.5	4.2	1.4
2	3.8	>20	4.5	6.5	5.2	6.0	8.1	11.7
3	2.0	>20	3.5	5.4	3.8	2.6	4.8	1.5
4	4.8	>20	4.9	7.0	5.1	3.5	7.5	12.0
5	1.9	0.8	0.2	8.2	14.3	1.1	1.2	>20

[a]Results are expressed as ED_{50} values (μg/ml).

[b]Key: A, KB; B, Vincristine-resistant KB; C, Fibrosarcoma; D, Melanoma; E, Lung Cancer; F, Breast Cancer; G, Colon Cancer; H, P-388.

with the fibrosarcoma cell line available to our investigation, implying selectivity. However, it should be noted that this compound is not as potent as many other quassinoids in the KB test system (11).

The antimalarial activity of eurycomanone [**5**] has been reported before (8) and was confirmed in our laboratory. In the present investigation, 7-methoxy-β-carboline-1-propionic acid [**7**] was also found to demonstrate a very weak antimalarial response with cultured *P. falciparum*. This result can be compared to harmine and harmaline alkaloids having structures similar to that of compound **7** that have been found to be active as antiprotozoal agents (23).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's, optical rotations, and uv, ir, nmr, and high and low resolution ms were measured as described previously (1). ^1H-^{13}C COLOC nmr experiments were performed on a Varian XL-300 instrument, using a standard Varian pulse program ($^3J_{CH} = 8$ Hz). ^1H-^1H NOESY nmr experiments were performed on a General Electric GN-500 nmr instrument.

PLANT MATERIAL.—The roots of *E. longifolia* (100 g) were collected in Samarinda, Kalimantan, Indonesia and identified by one of us (K.P.). A voucher specimen (no. 9279) has been deposited in the Herbarium of the Department of Biology, Bandung Institute of Technology, Bandung, Indonesia.

SOLVENT EXTRACTION.—The air-dried, milled plant material (100 g) was extracted by percolation (room temperature three times overnight) with MeOH to afford an initial MeOH extract (1.2 g) on removal of solvent in vacuo (P-388, ED_{50} 16 μg/ml).

ISOLATION AND CHARACTERIZATION OF COMPOUNDS **1–7**.—A portion of the MeOH-soluble extract (1.1 g) was subjected to cc over Si gel (400 g, 63–200 μm), using $CHCl_3$ containing increasing amounts of MeOH, as solvents. Altogether, nine fractions (F002–F010) were collected and combined on the basis of similar tlc profiles. Fraction F003 (80 mg, P-388, ED_{50} 12 μg/ml) was further purified by chromatography over Si gel (100 g) with $CHCl_3$-MeOH (99:2) as solvent to afford, in turn, 9-methoxycanthin-6-one [**1**] and 9-methoxycanthin-6-one-*N*-oxide [**2**]. Compound **1**, which was recrystallized from Me_2CO as yellow crystals (12 mg, 0.012% w/w) exhibited mp 181–183° [lit. (12) 178–180°] and spectroscopic data (uv, ir, ^1H-nmr, ms) comparable to literature values (11,12). Tlc of **1** and an authentic compound previously assigned as 10-methoxycanthin-6-one isolated from *S. multiflora* (14), using $CHCl_3$-MeOH (98:2) as the solvent system, indicated further their co-identity (R_f 0.50). Unambiguous ^{13}C-nmr assignments for **1** are provided in Table 1.

Compound **2** (mp 238–240°) precipitated as a reddish-yellow powder from MeOH (10 mg, 0.010% w/w). In $CHCl_3$, the solution gave a very intense green-yellow fluorescence. Compound **2** exhibited uv λ max ($CHCl_3$) (log ε) 242 (3.01), 286 (4.19), 293 (4.27), 360 (3.80), 422 nm (3.75); ir ν max (KBr) 1700, 1669, 1494, 1421, 1275, 1030, 844 cm^{-1}; ^1H nmr see Table 2; ^{13}C-nmr see Table 1; eims (70 eV) *m/z* $[M]^+$ 266 (100%), 251 (37), 250 (47), 236 (9), 223 (19), 207 (20), 195 (20), 179 (39), 153 (23); hreims 266.0690 (calcd for $C_{15}H_{10}N_2O_3$, 266.0689).

Conversion of compound **1** to **2**. Compound **1** (5 mg) was dissolved in 1 ml $CHCl_3$, with 5 mg *m*-chloroperbenzoic acid in 1 ml $CHCl_3$ added. After 24 h at room temperature the reaction mixture was

washed with 5 ml of 5% aqueous $NaHCO_3$ solution. The organic layer was separated and crystallized from $CHCl_3$-MeOH (1:1) to afford compound **2** (4.5 mg, 90% yield).

Fraction F006 (180 mg, P-388, ED_{50} 8 μg/ml) was further purified by chromatography over Si gel (200 g), commencing with $CHCl_3$-MeOH (96:4), then by elution with more polar $CHCl_3$/MeOH mixtures. This led to the isolation, in turn, of 9-hydroxycanthin-6-one [**3**] (24 mg, 0.024% w/w), 9-hydroxycanthin-6-one-*N*-oxide [**4**] (16 mg, 0.016% w/w), eurycomanone [**5**] (62 mg, 0.062% w/w), β-carboline-1-propionic acid [**6**] (24 mg, 0.024% w/w), and 7-methoxy-β-carboline-1-propionic acid [**7**] (18 mg, 0.018% w/w). These isolates were characterized as follows.

Compound **3**, yellow needles, mp 285–286° (dec); uv λ max (MeOH) (log ε) 238 (sh, 4.20), 274 (4.20), 310 (4.05), 352 nm (4.15); (+ NaOH), 236 (sh, 4.25), 294 (4.15), 316 (sh, 4.10), 355 (4.00), 428 nm (3.90); ir ν max (KBr) 3400–3600, 1700, 1663, 1653, 1636, 1570, 1559, 1457, 1355, 1320, 1285, 1250, 1155, 1064, 986, 929, 850, 835, 635 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 1; eims (70 eV) *m/z* $[M]^+$ 236 (100), 224 (10), 209 (8), 208 (50), 197 (24), 179 (10), 150 (58), 123 (22), 118 (5); hreims 236.0590 (calcd for $C_{14}H_8N_2O_2$, 236.0585). When compound **3** (5 mg) was methylated with CH_2N_2 using the Diazald Kit (Aldrich, Milwaukee, Wisconsin), 4 mg of a product was obtained that was identical (mmp, ^1H nmr, uv, ir, ms) to compound **1**. Compound **3** and an authentic compound previously assigned as 10-hydroxycanthin-6-one isolated from *S. multiflora* exhibited the same Si gel tlc migration data (R_f 0.40), using $CHCl_3$-MeOH (95:5).

Compound **4**, red needles, mp 248–250° (dec.), in MeOH gave a very intense yellow-green fluorescence. Compound **4** exhibited uv λ max (MeOH) (log ε) 230 (4.52), 289 (3.50), 360 (3.29), 417 nm (3.08); (+ NaOH) 230 (4.52), 310 (3.44), 348 (sh, 3.60), 4.83 nm (3.68); ir ν max (KBr) 3400–3500 (OH), 1686, 1606, 1502, 1471, 1280, 1245, 846 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 1; eims (70 eV) *m/z* $[M]^+$ 252 (100), 251 (5), 237 (8), 236 (48), 235 (3), 225 (1), 224 (8), 208 (21), 179 (14), 169 (10); hreims 252.0540 (calcd for $C_{14}H_8N_2O_3$, 252.0534). When compound **4** (3 mg) was methylated with CH_2N_2 using the Diazald Kit, 2 mg of a product was obtained that was identical (mmp, ^1H nmr, uv, ir, ms) to compound **2**.

Compound **5**, eurycomanone, obtained as white cubic crystals, exhibited mp 254–255°, $[\alpha]^{25}D$ +32° (*c* = 0.5, pyridine) [lit. (6) mp 253–255°, $[\alpha]^{25}D$ 32° (*c* = 1, pyridine)]; and spectroscopic data (uv, ir, ^1H nmr, ms) comparable to literature values (6,8). ^{13}C nmr (pyridine-d_5) δ 197.40 (C-2), 173.82 (C-16), 162.53 (C-4), 147.99 (C-13), 126.03 (C-3), 119.29 (C-18), 109.55 (C-11), 84.50 (C-1), 80.96 (C-12), 79.34 (C-14), 75.83 (C-7), 71.75 (C-15), 67.64 (C-30), 52.57 (C-8), 47.69 (C-9), 45.91 (C-10), 42.18 (C-5), 25.69 (C-6), 22.41 (C-17), 10.39 (C-19).

Compound **6**, obtained as white needles, exhibited mp 215–216° [lit. (21) 216°] and spectroscopic data (uv, ir, ms) comparable to literature values (21). ^1H nmr (DMSO-d_6) δ 11.64 (1H, s, N*H*), 8.24 (1H, d, *J* = 5 Hz, H-3), 8.18 (1H, dd, *J* = 8, 1.5 Hz, H-5), 7.94 (1H, d, *J* = 5 Hz, H-4), 7.60 (1H, dd, *J* = 8, 1.5 Hz, H-6), 7.53 (1H, dd, *J* = 8, 1.5 Hz, H-8), 7.21 (1H, dd, *J* = 8, 1.5 Hz, H-7), 3.34 (2H, t, *J* = 7.5 Hz, H-2′), 2.87 (2H, t, *J* = 7.5 Hz, H-1′); ^{13}C nmr (DMSO-d_6) δ 174.07 (C-3′), 144.03 (C-9), 140.36 (C-1), 137.29 (C-3), 133.98 (C-12), 127.77 (C-6), 111.89 (C-8), 31.29 (C-1′), 28.04 (C-2′).

Compound **7**: white needles; mp 160–162°; uv λ max (MeOH) (log ε) 207 (3.68), 249 (4.90), 326 (3.52), 358 nm (sh, 3.70); ir ν max (KBr) 3400–3500, 1700, 1685, 1653, 1636, 1628, 1624, 1617, 1560, 1539, 1457 cm^{-1}; ^1H nmr (DMSO-d_6) δ 11.42 (1H, N*H*), 8.18 (1H, d, *J* = 5 Hz, H-3), 8.05 (1H, d, *J* = 8 Hz, H-5), 7.82 (1H, d, *J* = 5 Hz, H-4), 7.01 (1H, d, *J* = 2.5 Hz, H-8), 6.83 (1H, dd, *J* = 8, 2.5 Hz, H-6), 3.88 (3H, s, O*CH*$_3$), 3.31 (2H, t, *J* = 7 Hz, H-2′), 2.85 (2H, t, *J* = 7 Hz, H-1′); ^{13}C nmr (DMSO-d_6) δ 174.22 (C-3′), 160.12 (C-7), 143.26 (C-1), 142.01 (C-9), 137.55 (C-3), 134.08 (C-12), 127.44 (C-11), 122.62 (C-5), 114.77 (C-10), 112.19 (C-4), 109.14 (C-6), 94.57 (C-8), 55.32 (O*CH*$_3$), 31.38 (C-1′), 27.97 (C-2′); eims (70 eV) *m/z* $[M]^+$ 270 (14%), 153 (16), 252 (100), 151 (33), 236 (8), 226 (15), 225 (55), 224 (74), 223 (65), 181 (33); hreims, 270.1000 (calcd for $C_{15}H_{14}N_2O_3$, 270.1001).

CYTOTOXIC ASSAYS.—Extracts, fractions, and compounds were evaluated for cytotoxic potential as described previously (24–26).

ANTIMALARIAL ASSAYS.—Cultures of *P. falciparum* (chloroquine-sensitive strain D-6 derived from CDC Sierra Leone, and chloroquine-resistant strain W-2 derived from CDC Indochina III) were maintained in human erythrocytes as described previously (27). Parasites were inoculated into type A+ human erythrocytes at a hematocrit of 6% in culture medium [RPMI-1640 (GIBCO Laboratories, Grand Island, New York) supplemented with 32 mM $NaHCO_3$ (GIBCO), 35 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, Sigma Chemical, St. Louis, Missouri), and 10% heat inactivated human plasma type A+]. Parasitemia was maintained below 4% under an atmosphere of 5% O_2, 5% CO_2, and 90% N_2 in 25-cm^2 tissue culture flasks at 37°.

The antimalarial activity of test compounds was assessed with an in vitro radioisotope-incorporation method (28). A suspension (200 μl) of *P. falciparum*-infected red blood cells (0.5–1.0% parasitemia, 1.0%

cell hematocrit) was added to the wells of standard microtiter plates (No. 25861, Corning Glass Works, Corning, New York) containing various concentrations of test substance (25 μl). Each compound was assayed in triplicate over a concentration range of 7–5000 ng/ml. In addition, known antimalarial drugs (quinine, chloroquine, mefloquine, and artemisinin) were tested in each experiment over a concentration range of 0.3–250 ng/ml. Microtiter plates were then incubated for 24 h at 37° in a sealed chamber under an atmosphere of 5% CO_2, 5% O_2, and 90% N_2. After this incubation period, 0.5 μCi (25 μl) of generally labeled [^{3}H]-hypoxanthine (NET 177, New England Nuclear Research Products, Boston, Massachusetts) was added to each well, and the microtiter plates returned to the sealed chamber at 37° for an additional 18–24 h incubation period. The assay was terminated by harvesting the contents of each microtiter plate onto a glass fiber filter mat (No. 11731, Skatron Inc., Sterling, Virginia) using a Skatron Model 11021 semi-automatic cell harvester. Filters were dried, and the radioactivity from individual wells was excised from the filter mat and placed in 7-ml vials. Toluene-based scintillation cocktail (Econofluor, New England Nuclear Research Products) was added, and radioactivity was determined with a Beckman LS 5801 liquid scintillation counter. [^{3}H]-hypoxanthine incorporation due specifically to malarial parasites was obtained by subtracting the mean dpm of unparasitized red cells from all data, and the results were expressed as a percent of uninhibited, parasite-specific [^{3}H]-hypoxanthine incorporation. The concentrations of test substances and positive control compounds required to inhibit incorporation by 50% (IC_{50} values) were then calculated by linear regression and estimation analysis. The results are shown in Table 4.

TABLE 4. Evaluation of the Antimalarial Activity of Isolates **5** and **7**.[a]

Compound	*Plasmodium falciparum* strain	
	D-6	W-2
Chloroquine	1.9	74.7
Quinine	19.4	114.4
Mefloquine	9.5	4.0
Artemisinin	2.7	1.8
5	47.7	48.1
7	3144	2978

[a]Results are expressed as IC_{50} values (ng/ml).

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