In Vitro Antiplasmodial Activity of Extracts of *Tristaniopsis* Species and Identification of the Active Constituents: Ellagic Acid and 3,4,5-Trimethoxyphenyl-(6′-*O*-galloyl)-*O*-*β*-D-glucopyranoside

Luisella Verotta,*[,†] Mario Dell'Agli,[‡] Andrea Giolito,[†] Marco Guerrini,[§] Pierre Cabalion,[⊥] and Enrica Bosisio*[,‡]

Department of Organic and Industrial Chemistry and Institute of Pharmacological Sciences, University of Milan, Italy, Institute of Chemistry and Biochemistry "G. Ronzoni", Milano, Italy, and Institut de Recherche pour le Développement (IRD), Noumea, New Caledonia

Received June 19, 2000

Screening of plants from New Caledonia for antiplasmodial activity against *Plasmodium falciparum* revealed that methanolic extracts of the leaves and bark of *Tristaniopsis calobuxus*, *T. yateensis*, and *T. glauca* inhibited the growth of chloroquine-sensitive and -resistant clones. Ellagic acid and the new compound 3,4,5-trimethoxyphenyl-(6′-*O*-galloyl)-*O*-*β*-D-glucopyranoside were identified as the active constituents (IC_{50} 0.5 and 3.2 μM, respectively). The growth inhibition of both clones was comparable. The compounds showed negligible or very low cytotoxicity to human skin fibroblasts and Hep G2 cells when tested at concentrations ranging from 0.5 to 100 μM.

Malaria remains a major problem of public health worldwide (200 million people of which 1−2 million die each year), mainly due to an increase in parasite resistance to available drugs.[1] The search for new antimalarial drugs is essential and requires the identification of new biochemical targets for drug development.[2] Studies conducted in various laboratories have led to the discovery of several new targets, including the heme detoxification process, isoprenoid and phospholipid biosynthesis, nucleic acid metabolism, proteases, and others.[3]

Screening of plants collected in New Caledonia for anti-elastase activity led to the identification of some active species.[4] Elastase is a serine proteinase involved in a variety of inflammatory processes, including emphysema,[5] parodontosis,[6] and cystic fibrosis.[7] Proteinases have also been shown to be essential to a variety of viruses and other pathogenic microorganisms.[2] In particular, *Plasmodium falciparum* degrades hemoglobin by means of two aspartic proteinases (plasmepsin I and II) and one cysteine proteinase (falcipain), which can be potential targets for the search for new drugs.[2,3] Thus, we also evaluated the extracts with anti-elastase activity for antiplasmodial activity: D6 (chloroquine-sensitive, moderately mefloquine resistant) and W2 (chloroquine-resistant, mefloquine sensitive) clones of *Plasmodium falciparum*. Among the tested plants, *Tristaniopsis calobuxus* Brongniart & Gris (Myrtaceae) inhibited parasite growth in vitro. From the bark extract, two compounds with in vitro antiplasmodial activity were isolated and identified as ellagic acid and 3,4,5-trimethoxyphenyl-(6′-*O*-galloyl)-*O*-*β*-D-glucopyranoside (Figure 1).

Results and Discussion

Among the plants evaluated for antiplasmodial activity, the methanolic extract of the bark of *Tristaniopsis calobuxus* Brongniart & Gris gave positive results (Table 1). Following an activity-guided procedure, the crude extract was counterextracted and the activity was retained mainly in the ethyl acetate fraction (Table 2), which was chromatographed on Sephadex LH20 eluted with methanol. Three active fractions were separated (Table 2) and submitted to further fractionation, leading to the isolation of compounds A1−A5, B1−B4, AB1, and D1 (Table 3). From fraction 149−185, compound D1 was crystallized and showed an IC_{50} of 0.1 μg/mL against both clones. D1 was identified as ellagic acid. From fraction 122−148 was obtained C1, a mixture containing ellagic acid (IC_{50} 1.6 and 1.4 μg/mL against D6 and W2 clones, respectively), which was presumed to account for the observed biological activity.

From fraction 90−96, compound A3A was isolated as an amorphous powder and showed an IC_{50} of 1.7 and 1.6 μg/mL against D6 and W2 clones, respectively.

Its ^{1}H NMR spectrum (500 MHz, DMSO-d_6) showed the presence of two singlets at δ 6.93 and 6.28, respectively, each corresponding to two aromatic protons (quantitative spectra were obtained by recording the experiments with a recycle time of 45 s). The two shielded protons, characterized by an unexpectedly long relaxation time, allowed us to propose the presence of isolated aromatic protons in symmetrical relationship with the rest of the molecule, positioned in an electron donor environment. A signal consistent with a sugar anomeric proton was observed at δ 4.88 (d, J = 7.7 Hz). Signals for the other sugar protons were found at δ 4.54 (dd J = 12.0, 1.9 Hz, H-6′A) and δ 4.25 (dd, J = 12.0, 5.8 Hz, H-6′B); the remaining protons are part of a strong coupled system and resonate at δ 3.72 (1H, m), 3.32 (1H, m), 3.27 (1H, m), and 3.23 (1H, m). Two more singlets, corresponding to six and three protons, respectively, were observed at δ 3.62 and 3.55. The decoupled ^{13}C NMR spectrum, together with DEPT experiments, revealed the presence of five singlets: one carbonyl group at δ 166.0 and four aromatic quaternary carbons at δ 153.8 (1C, C-1), 138.5 (1C, C-4″), 132.9 (1C, C-4), and 119.6 (1C, C-1″); seven doublets at δ 108.7 (2C, C-2″ + 6″), 100.9 (1C, C-1′), 94.6 (2C, C-2+C-6), 76.2 (1C, C-3′), 74.0 (1C, C-5′), 73.3 (1C, C-2′), and 69.9 (1C, C-4′), respectively; one triplet at δ 63.7 (1C, C-6′); and two methoxy groups at

* Corresponding authors. (E.B.) Institute of Pharmacological Sciences, Via Balzaretti, 9, 20133 Milano, Italy. Tel: 39-02-20488372. Fax: 39-02-29404961. E-mail: enrica.bosisio@unimi.it. (L.V.) Department of Organic and Industrial Chemistry, Via Venezian 21, 20133 Milano, Italy. Tel 39-02-2363469. Fax: 39-02-2364369. E-mail: luisver@icil64.cilea.it.
† Department of Organic and Industrial Chemistry, University of Milan.
‡ Institute of Pharmacological Sciences, University of Milan.
§ Institute of Chemistry and Biochemistry "G. Ronzoni".
⊥ Institut de Recherche pour le Développement (IRD).

10.1021/np000306j CCC: $20.00
Published on Web 04/27/2001

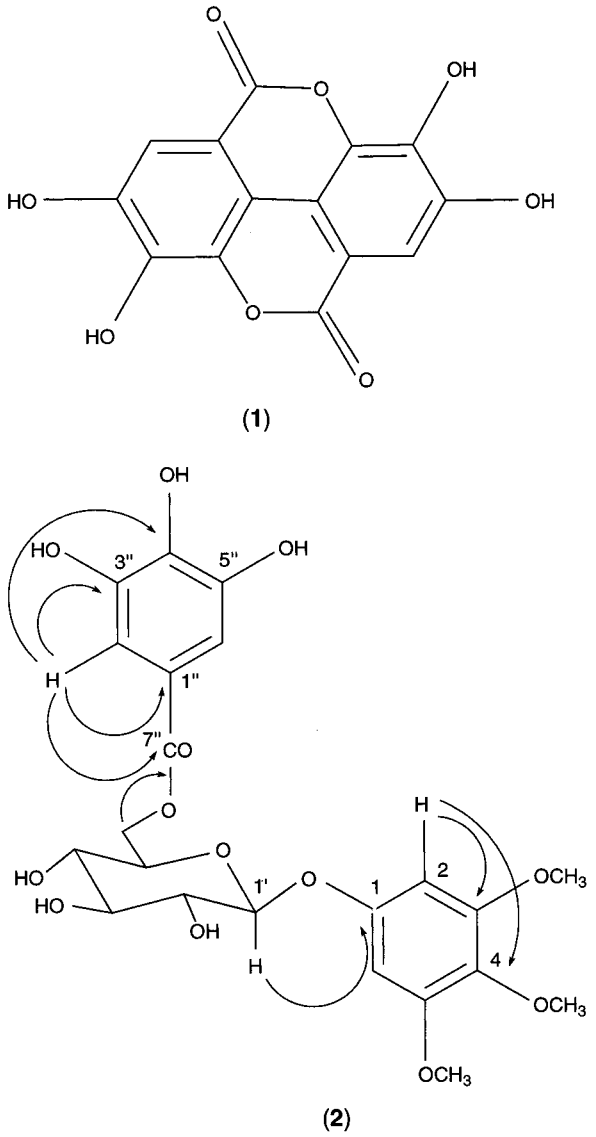

Figure 1. Ellagic acid (**1**) and 3,4,5-trimethoxyphenyl-(6′-*O*-galloyl)-*O*-*β*-D-glucopyranoside (**2**).

δ 60.3 (1C) and δ 55.9 (2C), respectively. Assignments were made possible through the HSQC and HMBC experiments.

The presence of an acylated phenolic *β*-D-glucoside was hypothesized. The acyl moiety is comprised of a galloyl residue esterified with the primary alcoholic hydroxy group of the sugar. The acylated monosaccharide is linked through an acetal bond to a trimethoxyphenol residue, as confirmed by HMBC experiments. NOE interactions between the aromatic protons at δ 6.28 and the anomeric proton, and between the same aromatic protons and the two methoxy groups at δ 3.62, indicated the presence of a 3,4,5-trimethoxyphenol glucoside.

The unambiguous structural assignment was made possible by chemical degradation. Treatment of the natural glucoside with NaOMe/MeOH yielded 3,4,5-trimethoxyphenol *β*-D-glucopyranoside, which was identified by comparison with a synthetic sample prepared by glucosidation of 3,4,5-trimethoxyphenol. Any attempt to selectively acylate the hydroxymethylene group of the sugar moiety in 3,4,5-trimethoxyphenol *β*-D-glucopyranoside using previously successful methods on similar phenolic glucosides failed.[8] It is postulated that 3,4,5-trimethoxyphenol can be derived biogenetically from oxidative decarboxylation of 3,4,5-trimethoxybenzoic acid. This is the first report of a 3,4,5-trimethoxyphenol glucoside from a natural source.[9]

This is also the first report of the inhibition of *Plasmodium* growth by ellagic acid. Biological properties of ellagic acid reported previously include antimutagenic and tumor chemopreventive activity.[10−16] It has previously been shown that in rats ellagic acid is partially absorbed, metabolized by intestinal flora, and excreted in bile and urine as glucuronide and glutathione conjugates.[17,18] In freshly isolated rat hepatocytes, gallic acid is converted to ellagic acid by autoxidation, and the conversion reduced gallic acid cytotoxicity.[19] A3A is less active as compared to ellagic acid. It may be speculated that this is due to lower bioavailability or the need to be metabolically activated. In addition, since galloyl and glucose moieties are devoid of activity (see Table 3, gallic acid inactive vs *Plasmodium* growth), then it would seem that only the trimethoxyphenol group is likely to contribute to the observed effect.

Although the isolated compounds are less potent than chloroquine (IC_{50} 2.54 and 58 ng/mL for D6 and W2, respectively) and mefloquine (3.98 and 1.36 ng/mL), it is noteworthy that the compounds are active against the resistant clone. In addition, interest in plant phenols as potential complementary agents has recently arisen because they have been shown to potentiate the in vitro effect of artemisinin.[20]

Three other *Tristaniopsis* species were also tested for antiplasmodial activity. Antiplasmodial activity was found in the leaves and bark of *Tristaniopsis glauca* Brongniart & Gris and *Tristaniopsis yateensis* J. W. Dawson, and in the leaves, branches, and root bark of *T. calobuxus* (Table 4).

To compare the effect on the parasite with toxicity to mammallian cells, the antiproliferative activities of the isolated compounds against human skin fibroblast and the hepatoma G2 line (Hep G2) were evaluated. A3A was not cytotoxic to either cell type at 100 μM, the highest concentration tested, while ellagic acid, at 100 μM, showed a slight cytotoxicity to Hep G2 and fibroblasts, with 9% and 20% decreases in proliferation, respectively.

These results suggest a negligible cytotoxic effect of the compounds on mammalian cells at the concentrations found to inhibit the parasite growth (IC_{50} 0.5 and 3.2 μM for ellagic acid and A3A, respectively), suggesting they are selectively toxic to the parasite.

Experimental Section

Plant Material. Plants were collected by Dudley Nicholls (DN), Institut de Recherche pour le Devéloppement (IRD), Noumea, and dried in an oven at 40 °C. Plant identity was verified at the herbarium of the IRD Center of Noumea, and voucher specimens were deposited at the Laboratory of Natural Substances of Biological Interest, Noumea, New Caledonia: *T. calobuxus* Brongniart & Gris (DN no. 17, collection June 19th, 1996, at Riviére des Pirogues, direction to Yaté); *Tristaniopsis yateensis* J.W. Dawson (DN no. 57, collection July 17th, 1997, at the Col de Yaté); *Tristaniopsis glauca* Brongniart & Gris (DN no. 58 collection July 17th, 1997, at Lac de Yaté).

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Si gel 60 (70−230 mesh, Merck) was used for open-column chromatography. Sephadex LH20 (Pharmacia Biotech) was used for gel filtration chromatography. Mass spectra were recorded on a VG Analytical 7070 EQ spectrometer at 70 eV in the electron impact and chemical ionization (NH_3) mode.

The HSCCC experiment was carried out with a centrifugal countercurrent chromatograph from Pharma-Tech Research

Table 1. Screening of Methanolic Plant Extracts for Antiplasmodial Activity

plant	yield,[a] % w/w	parasite clone	IC_{50}, ng/mL
Ximenia americana L. (Olacaceae) bark	22	D6	>50000
		W2	
Oncotheca balansae Baillon (Oncothecaceae) bark	7.9	D6	>50 000
		W2	
Cunonia montana Schlecter (Cunonaceae) bark	7.8	D6	>50 000
		W2	
Tristaniopsis calobuxus Brongniart & Gris (Myrtaceae) bark	7.1	D6	8970
		W2	7652
Amyema scandens Danser(Loranthaceae) leaves	10	D6	>50 000
		W2	
Guioa villosa Radlk (Sapindaceae) leaves	12	D6	>50 000
		W2	
Diospyros vieillardii (Hiern) Kostermans (Ebenaceae) leaves	15	D6	>50 000
		W2	
Breynia disticha J. R. & G. Foster (Euphorbiaceae) bark	22	D6	>50 000
		W2	
Trema orientalis L. (Ulmaceae) bark	16	D6	>50 000
		W2	

[a] Extraction recovery calculated on the dry material.

Table 2. Activity-Guided Fractionation of the Bark Extract of *T. calobuxus*

compound	parasite clone	IC_{50}, ng/mL
methylene chloride fraction	D6	7036
	W2	4297
ethyl acetate fraction	D6	1727
	W2	1112
aqueous fraction	D6	>50 000
	W2	
Sephadex LH 20 fractions		
20−89	D6	>5000
	W2	
90−96	D6	2600
	W2	1822
97−105	D6	>5000
	W2	
106−121	D6	>5000
	W2	
122−148	D6	1508
	W2	1254
149−185	D6	181
	W2	113
186−end	D6	>5000
	W2	

Table 3. Antiplasmodial Activity of Isolated Compounds

compound[a]	parasite clone	IC50, ng/mL
A1	D6	>5000
	W2	
A2	D6	2245
	W2	2651
A3A	D6	1747
	W2	1585
A4	D6	>5000
	W2	
A5	D6	>5000
	W2	
B1	D6	>5000
	W2	
B2	D6	>5000
	W2	
B3	D6	>5000
	W2	
B4	D6	>5000
	W2	
AB1	D6	>5000
	W2	
D1	D6	145
	W2	103
chloroquine	D6	2.54
	W2	58
mefloquine	D6	3.98
	W2	1.36

[a] Compounds A5, B1 and AB1 were identified as p-OH benzoic acid, gallic acid and 3,4-di-OH benzoic acid, respectively; Compound **A3A** was identified as 3,4,5 trimethoxyphenol-(6′-O-galloyl)-O-β-D-glucopyranoside; compound **D1** is ellagic acid.

Corp. (Baltimore, MD, model CCC 1000). It was equipped with a SSI (Scientific Systems) liquid chromatography pump (model 300), a high-speed countercurrent chromatograph electronic controller PTRC, and an injection valve (Rheodyne). The HSCCC experiments were performed with a two-phase solvent system composed of (1) $CHCl_3$−MeOH−H_2O, 5:6:4, or (2) $CHCl_3$−MeOH−i-PrOH−H_2O, 5:6:1:4.

The two-phase system was prepared by thoroughly mixing the solvents in a separatory funnel at room temperature and leaving it to settle for 12 h. The column was entirely filled with the stationary phase (lower phase) at a flow rate of 9 mL/min, after the mobile phase (upper phase) was pumped into the inlet of the column at a rotation speed of 1040 rpm and a flow rate of 1 mL/min. The elution mode was tail to head. The equilibration volume was 90 mL for two-phase solvent system 1 and 60 mL for two-phase solvent system 2. The sample was filtered on a Millex HV filter (0.45 μm, Millipore).

Si gel 60 F_{254} (Merck) TLC was eluted with the organic phase of the mixture $CHCl_3$−MeOH−i-PrOH−H_2O (5:6:1:4). Spots were revealed by UV absorption (254 nm) and by spraying with $FeCl_3$ or H_2SO_4 followed by heating. NMR: the sample was prepared by dissolving 8 mg in 0.6 mL of DMSO-d_6 (99.96% D); 500 MHz spectra were recorded on a Bruker AMX500 spectrometer, equipped with a 5 mm inverse broad-band probe with a shielded *z*-axis gradient, at 313 K. ^{1}H and ^{13}C chemical shifts are referenced to the solvent signal (2.49 ppm ^{1}H and 49.5 ppm ^{13}C). The DQF-COSY spectrum was acquired using 16 scans per series in 1K × 512W data points, with zero filling in F1, and a sine function was applied before Fourier transformation. ^{1}H/^{13}C chemical shift correlations (HSQC) were performed using *z*-gradients for coherence selection. An HSQC spectrum was collected with decoupling during the acquisition period in phase sensitivity-enhanced pure absorption mode;[21] the matrix size was 1K × 512 points, and the experiment was zero-filled to 2K × 1K and multiplied with shifted sine-bell-squared prior Fourier transformation. The HMBC spectra[22] were acquired using 128 scans per series in 1K × 256W data points. The HMBC spectra were optimized for a $^nJ_{C-H}$ of 8 Hz, with $n = 2-4$. The experiment was zero-filled and multiplied with sine-bell prior to Fourier transformation. The recycle delay for all 2D spectra was about 3 s.

Extraction and Isolation. For initial assays, the plant material was powdered, delipidized with petroleum ether at 40−60 °C, and extracted twice with methanol (5 g/30 mL). The extracts were filtered, concentrated in vacuo, and weighed for the determination of the w/w yield (Table 1). For purification and product isolation, 300 g of *T. calobuxus* bark was delip-

Table 4. Antiplasmodial Activity of Methanolic Extracts of *Tristaniopsis* spp.

plant extract		parasite clone	IC_{50}, ng/mL
T. calobuxus Brongniart & Gris	leaves	D6	7605
		W2	4058
	branches	D6	15 960
		W2	7895
	root bark	D6	15 284
		W2	7945
T. yateensis J.W. Dawson	bark	D6	15 682
		W2	8024
	leaves	D6	7453
		W2	4221
T. glauca Brongniart & Gris	bark	D6	14 800
		W2	7529
	leaves	D6	7864
		W2	4476

idized and extracted twice with 300 mL of methanol (recovery 11%). The crude extract was further fractionated in methylene chloride (yield 11%), ethyl acetate (yield 5%), and water-soluble phases. Extracts were kept at −20 °C until use and dissolved in DMSO for the biological assays.

The ethyl acetate extract (1.7 g) was purified on Sephadex LH20 (2.5 × 88 cm) eluted with MeOH at a flow rate of 14 mL/h. A total of 214 fractions (6 mL each) were collected. Based on the TLC similarities, identical fractions were combined to give a total of seven fractions: 20−29 = 240 mg; 90−96 = 79 mg; 97−105 = 194 mg; 106−121 = 113 mg; 122−148 = 205 mg; 149−185 = 337 mg; and 186−214 = 478 mg.

Fraction 90−96 (79 mg) was submitted to countercurrent chromatography using the solvent system $CHCl_3$−MeOH−H_2O, 5:6:4 (mobile phase = upper phase). Six fractions were obtained: A1 (8 mg), A2 (12.5 mg), A3 (15 mg), A4 (2.1 mg), A5 (11.9 mg), and a fraction that was further purified by silica gel ($CHCl_3$−MeOH 85:15) chromatography to afford AB1 (5 mg).

A5 and AB1 were identified as *p*-hydroxybenzoic acid and 3,4-dihydroxybenzoic acid, respectively, by comparison of their spectral data (NMR and MS spectra) with commercial samples.

A3 was purified on silica gel ($CHCl_3$−MeOH, 85:15) to yield 6 mg of compound A3A **(**0.002%), 3,4,5-trimethoxyphenyl-(6′-*O*-galloyl)-*O*-*β*-D-glucopyranoside, as a white amorphous powder: $[\alpha]^{25}_D$ −23.4° (*c* 0.19, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) *δ* 6.93 (2H, bs, H-2″, H-6″), 6.28 (2H, bs, H-2, H-6), 4.88 (1H, d, *J* = 7.7 Hz, H-1′), 4.45 (1H, dd, *J* = 12.0, 1.9 Hz, H-6′A), 4.29 (1H, dd, *J* = 12.0, 5.8 Hz, H-6′B), 3.72 (1H, m, H-5′), 3.62 (6H, s, 2 × OCH_3), 3.55 (3H, s, OCH_3), 3.32 (1H, m, H-3′), 3.27 (1H, m, H-4′), 3.23 (1H, m, H-2′); ^{13}C NMR (75.5 MHz, DMSO-d_6) *δ* 166.0 (s, C-7″), 153.8 (s, C-1), 153.3 (s, C-3, C-5), 145.6 (s, C-3″, C-5″), 138.5 (s, C-4″), 132.9 (s, C-4), 119.6 (s, C-1″), 108.7 (d, C-2″,6″), 100.9 (d, C-1′), 94.6 (d, C-2, C-6), 76.2 (d, C-3′), 74.0 (d, C-5′), 73.3 (d, C-2′), 69.9 (d, C-4′), 63.7 (t, C-6′), 60.3 (q, OCH_3), 55.9 (q, OCH_3); CIMS (NH_3, positive) *m*/*z* 516 $[MNH_4]^+$, 364 $[MNH_4 - C_7H_5O_4]^+$, 347 $[M-C_7H_5O_4]^+$, 202 [364 − $C_5H_{10}O_5]^+$, 185 [347 − $C_5H_{10}O_5]^+$; CIMS (NH_3, negative) *m*/*z* 497 $[M - H]^-$, 169 $[C_8H_9O_4]^-$; EIMS (70 eV) *m*/*z* 346 (5), 184 (100), 169 (49).

Fraction 97−105 (215 mg) was submitted to CCC using the solvent system $CHCl_3$−MeOH−i-PrOH−H_2O, 5:6:1:4 (mobile phase = upper phase). Eight fractions were obtained, of which B1 (150 mg, 0.04% based on dry material) was identified as gallic acid by comparison of its spectral data with those of a commercial sample.

Fraction 149−185 (337 mg) precipitated a residue (D1 = 125 mg) that was identified as ellagic acid. Additional ellagic acid was also isolated from the mother liquor (D2 = 195 mg).

Fraction 122−148 (295 mg) was counterextracted between BuOH−n-PrOH−H_2O, 2:1:3, and the organic phase (C1 = 142 mg) contained additional ellagic acid.

Hydrolysis of A3A. A 1.5 mg sample of A3A in MeOH (0.2 mL) was treated with NaOMe−MeOH (0.3 mL) for 3 h at room temperature. The reaction mixture was then stirred with a Dowex 50 W resin (15 mg), filtered, and evaporated to dryness. One milligram of 3,4,5-trimethoxyphenol-*β*-D-glucopyranoside was obtained.

Synthesis of 3,4,5-Trimethoxyphenol. A 3.5 g sample of 3,4,5-trimethoxyanilin was added to a solution of 50% H_2SO_4 (74 mL), under nitrogen, with stirring. At 0 °C, a solution of sodium nitrite (1.58 g/28 mL of water) was added dropwise, and then a solution of $CuSO_4$·$3H_2O$ (9.8 g/53 mL of water) was added. The mixture was stirred at room temperature for 30 min and then warmed at 60 °C for 45 min. The solution was extracted with CH_2Cl_2 (4 × 100 mL). The organic phases were dried and evaporated to dryness (2.4 g). The crude material was purified by Si gel (125 g) eluted with $CHCl_3$−EtOAc, 9:1 (3.1 L). A total of 140 fractions (20 mL each) were collected. Fractions 65−130 (1.7 g) were crystallized (i-Pr)$_2$O, to yield 0.95 g of 3,4,5-trimethoxyphenol, mp 142 °C [lit.[23] mp 145−6 °C]; ^{1}H NMR ($CDCl_3$, 200 MHz) *δ* 6.18 (2H, s, H-2+H-6), 4.80 (s, OH), 3.83 (9H, s, OCH_3); CIMS *m*/*z* 184 $[M]^+$ (68), 169 (100).

Synthesis of 3,4,5-Trimethoxyphenol-*β*-D-glucopyranoside. 3,4,5-Trimethoxyphenol (564 mg) in 1.25 M NaOH (4.1 mL) was added to a solution of triethylbenzylammonium bromide (TEBA) (39 mg) and α-bromotetraacetylglucose (1.4 g) in $CHCl_3$ (8 mL). The mixture was stirred at 70 °C for 20 h. Water was added (40 mL), and the mixture was extracted with $CHCl_3$ (3 × 40 mL) and dried with Na_2SO_4. After evaporation under vacuum, 1.53 g of an oily material was obtained and purified over silica gel (230 g) eluted with petrol−EtOAc 7:3 (1 L). A total of 200 fractions of 5 mL each were collected. Fractions 141−181 were pooled and taken to dryness, to afford 596 mg of 3,4,5-trimethoxyphenoltetraacetyl-*β*-D-glucopyranoside (38%).

3,4,5-Trimethoxyphenoltetraacetyl-*β*-D-glucopyranoside (500 mg) in MeOH (40 mL) was treated with 0.2 M NaOMe−MeOH (29 mL) for 3 h at room temperature. The mixture was neutralized with Dowex 50 W (5 g) and filtered. After solvent evaporation, 315 mg of crude material was obtained, which, after crystallization (i-PrOH−MeOH, 1:1), afforded 290 mg of 3,4,5-trimethoxyphenol-*β*-D-glucopyranoside: mp 204 °C; $[\alpha]^{25}_D$ −78° (*c* 1, MeOH); ^{1}H NMR (DMSO-d_6, 200 MHz) *δ* 6.40 (2H, bs, H-2+H-6), 4.81 (1H, d, H1′), 3.74 (6H, s, OCH_3), 3.64 (3H, s, OCH3), 3.32 (1H, m), 3.27 (1H, m), 3.23 (1H, m); ^{13}C NMR (25.4 MHz, DMSO-d_6) *δ* 154.0 (s, C-1), 153.3 (s, C-3, C-5), 132.5 (s, C-4), 101.1 (d, C-1′), 94.4 (d, C-2, C-6), 77.4 (d, C-5′), 76.9 (d, C-3′), 73.3 (d, C-2′), 70.2 (d, C-4′), 61.0 (t, C-6′), 60.2 (q, OCH_3), 55.8 (q, OCH_3); CIMS *m*/*z* 346 $[M]^+$ (5), 184 (100), 169 (63).

In Vitro Test for Antiplasmodial Activity. Material obtained at each purification step was tested for antiplasmodial activity against *P. falciparum* clones CDC/Sierra Leone I (D6: chloroquine sensitive, moderately mefloquine resistant) and CDC/Indochina III (W2: chloroquine resistant, mefloquine sensitive). Tests were performed at the Walter Reed Army Institute of Research (WRAIR) under a grant from the UNDP/World Bank/WHO Special Programme for Research and Training for Tropical Diseases (TDR). The method used was a modification of the procedures described previously.[24−26] The system is limited to the assessment of intrinsic activity against erythrocytic asexual blood stages. Chloroquine and mefloquine were used as reference compounds. IC_{50} for chloroquine was 2.54 and 58 ng/mL against D6 and W2, respectively. IC_{50} for mefloquine was 3.98 and 1.36 ng/mL against D6 and W2, respectively.

Cytotoxicity Assay. Cytotoxicity was evaluated in human dermal fibroblasts from child skin biopsies and Hep G2 cell (ATCC, Rockville, MD) lines. Cell proliferation was followed by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) test.[27] Fibroblasts (2 × 10^4/ml) were grown in DMEM (Dulbecco's modified Eagle's medium) containing 10% fetal calf serum, 1% penicillin/streptomycin, and 1% L-glutamine. Hep G2 (25 × 10^4/mL) were cultivated in DMEM containing 10% fetal calf serum, 1% penicillin/streptomycin, 1% L-glutamine, and 1% nonessential amino acids. The compounds to be tested were added to culture medium dissolved in DMSO or methanol, 1 day after plating. The final concentration of the vehicle in control and test medium was

1% of the incubation volume. The effect of compounds on cell proliferation was assayed at concentrations ranging from 0.5 to 100 μM, at 24 and 48 h after treatment. Each concentration was tested in quadruplicate in two separate experiments.

Acknowledgment. The authors are indebted to Dr. P. Olliaro (TDR, WHO) for providing facilities for the biological assays and for helpful discussion. Fibroblasts were a generous gift of Prof. Abatangelo, University of Padova, Italy. This work was supported by funds from the Ministry of the University and Scientific and Technological Research (MURST 40% and 60%).

References and Notes

(1) Wernsdorfer, G.; Wernsdorfer W. H. In *Malaria*; Wernsdorfer W. H., McGregor Sir I, Eds.; Churchill Livingstone: Edinburgh, 1992; Vol. 2; Chapter 47, pp 1421−71.
(2) Olliaro, P. L.; Gottlieb, M.; Wirth, D. F. *Parasitol. Today* **1996**, *12*, 413−14.
(3) Olliaro, P. L.; Yuthavong, Y. *Pharmacol. Ther.* **1999**, *81*, 91−110.
(4) Bosisio, E.; Mascetti, D.; Cabalion, P. *Pharm. Biol.* **2000**, *38*, 18−24.
(5) Janoff, A. *Am. Rev. Respir. Dis.* **1985**, *132*, 417−33.
(6) Giannopoulou, C.; Andersen, E.; Demeurisse, C.; Cimasoni, G. *J. Dent. Res.* **1992**, *71*, 359−63.
(7) Wiedow, O.; Streit, V.; Christophers, E.; Ständer, M. *Hautarz* **1989**, *40*, 518−22.
(8) Verotta, L.; Orsini, F.; Pelizzoni, F.; Torri, G.; Rogers, C. B. *J. Nat. Prod.* **1999**, *62*, 1526−31.
(9) While this paper was in preparation, a communication by Yun et al. (Yun, B.-S.; et al. *Arch. Pharmacal. Res.* **2000**, *23* (2), 147−50) appeared which reports the isolation of 3,4,5-trimethoxyphenyl-(6′-*O*-galloyl)-*O*-*β*-D-glucopyranoside from *Eucalyptus globulus*. No spectroscopic or physicochemical properties are here reported, because the authors refer to data reported in the literature (Ishimaru, K.; et al. *Phytochemistry* **1987**, *26* (4), 1147−1152). This communication again refers to another paper (Nonaka, G. I.; et al. *Chem Pharm. Bull.* **1982**, *30* (6), 2061−2067), where the authors describe the isolation and structural elucidation of a different compound, namely, 2,4,6-trimethoxyphenol 1-*O*-*β*-D-glucopyranoside.
(10) Wood, A. W.; Huang, M. T.; Chang, R. L.; Newmark, H. L.; Lehr, R. E.; Yagi, H.; Sayer, J. M.; Jerina, D. M.; Conney, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 5513−7
(11) Becker, C. G.; Hajjar, D. P.; Hefton J. M. *Am. J. Pathol.* **1985**, *120*, 1−5.
(12) Stoner, G. D.; Mukhtar, H. *J. Cell. Biochem.* **1995**, *22*, 169s−80s.
(13) Castonguay, A.; Boukharta, M.; Teel, R. W. *Chem. Res. Toxicol.* **1998**, *11*, 1258−64.
(14) Kaul, A.; Khanduja K. L. *Nutr. Cancer* **1998**, *32*, 81−5.
(15) Loarca-Pina, G.; Kuzmicky, P. A.; de Mejia, E. G.; Kado, N. Y. *Mut. Res.* **1998**, *398*, 183−7.
(16) Khanduja K. L.; Gandhi R. K.; Pathania, V.; Syal, N. *Food. Chem. Toxicol.* **1999**, *37*, 313−8.
(17) Teel R. W. *Cancer Lett.* **1987**, *34*, 165−71.
(18) Doyle, B.; Griffith, L. A. *Xenobiotica* **1980**, *10*, 247−56.
(19) Nakagawa, Y.; Nakajima k.; Tayama S.; Moldeus P. *Mol. Pharmacol.* **1995**, *47*, 1021−7.
(20) Elford, B. C.; Roberts M. F.; Phillipson J. D.; Wilson R. J. *Trans. R. Soc. Trop. Med. Hyg.* **1987**, *81*, 434−36.
(21) Schleucher, J.; Sattler, M.; Griesinger, C. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1489−1491.
(22) Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285−4294.
(23) Kosuge K.; Mitsunaga, K.; Koike, K.; Ohmoto, T. *Chem. Pharm. Bull.* **1994**, *42*, 1669−1671.
(24) Chulay, J. D.; Haynes, D. E.; Diggs, C. L. *Exp. Parasitol.* **1983**, *55*, 138−146.
(25) Desjardins, R. E.; Canfield, C. J.; Haynes, D. E.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710−718.
(26) Milhous, W. K.; Weatherly, N. F.; Bowdre, J. H.; Desjardins, R. E *Antimicrob. Agents Chemother.* **1985**, *27*, 525−530.
(27) Denizot, F.; Lang, R. *J. Immunol. Methods* **1986**, *89*, 271−7.

NP000306J