Synthesis and Antimalarial Activity of (+)-Deoxoartemisinin

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(+)-Deoxoartemisinin (**2**), a new and more active antimalarial agent, was successfully prepared from artemisinin in one step using $NaBH_4$ and $BF_3{\cdot}Et_2O$ in THF. (-)-Deoxodeoxyartemisinin (**5**), a potential metabolite of deoxoartemisinin, was also prepared either from **2** or from artemisinic acid. **2** shows 8-fold increased antimalarial activity in vitro against chloroquine-resistant malaria as compared to artemisinin (1). Compound **2** possesses superior in vivo antimalarial activity to 1.

Malaria is the number one infectious disease in the world today. Worldwide, over 2 million people die each year from malaria. This shocking reality is due largely to the emergence of drug resistant strains of *Plasmodium falciparum*. Artemisinin (Qinghaosu)[5] is an unusual antimalarial drug with a biologically active cyclic peroxide function. It has been shown to be a fast-acting, safe, and effective drug against chloroquine-resistant and sensitive strains of *P. falciparum*.[6] Due to the low solubility of artemisinin in water or oils and the unrealistically large doses of the drug required for therapy of malaria patients, a more ideal drug with enhanced antimalarial activity and improved physical and bioavailability properties is an urgent need to treat chloroquine-resistant malaria.

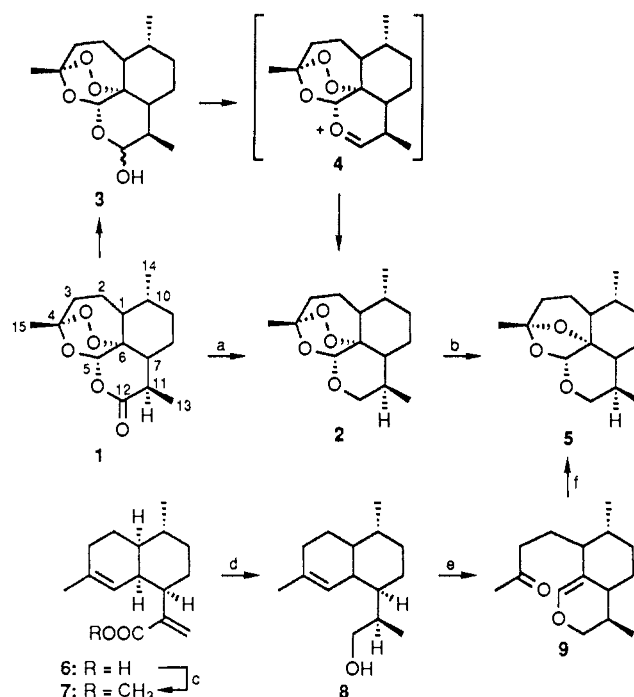
In continuation of our efforts[7] to find new antimalarial agents in this class of compounds,[8-14] we decided to prepare deoxoartemisinin (**2**). It is a new, nonnaturally occurring compound devoid of the carbonyl function at C-12 while the biologically active endoperoxide, an indispensable moiety for antimalarial activity,[6] is retained.

This paper reports the first successful synthesis and in vitro antimalarial activity of (+)-deoxoartemisinin (**2**) and (-)-deoxodeoxyartemisinin (**5**) and in vivo antimalarial activity of (+)-deoxoartemisinin (**2**).

Chemistry

Due to its structural complexity and the presence of the chemically sensitive lactone and peroxide bridge of artemisinin (**1**), difficulty for the direct reduction of **1** into **2** was anticipated. It was expected that this conversion would require at least three steps [i.e. reduction of **1** into the dihydroartemisinin **3**,[15] formation of oxonium ion **4**, and, finally, reduction of the reactive oxonium ion **4** into **2** (Scheme I)]. A practical and useful process to afford deoxoartemisinin (**2**) and eliminate an otherwise long process to remove the carbonyl function at C-12 of ar-

Scheme I[a]



[a] Reagents and conditions: (a) $NaBH_4$, $BF_3{\cdot}Et_2O$, THF, 0 °C for 1 h then reflux; (b) 5% $Pd/CaCO_3$, EtOH, room temperature for 1 h, *p*-TsOH, toluene; (c) CH_2N_2, Et_2O, room temperature; (d) $LiAlH_4$, $NiCl_2{\cdot}6H_2O$, Et_2O, room temperature for 3 h; (e) O_3, CH_2Cl_2, -78 °C for 2.5 h, *p*-TsOH, room temperature for 2 h; (f) *m*-CPBA, $CHCl_3$, 0°C for 2 h.

Table I. In Vitro Antimalarial Activity (IC_{50}, ng/mL) of Artemisinin-Related Drugs

antimalarial drug	clone of *P. falciparum* W-2[a]	D-6[b]
artemisinin (1)	1.21	2.33
dihydroartemisinin (3)	0.04	0.11
deoxoartemisinin (2)	0.15	0.58
β-arteether	0.16	0.87
deoxodeoxyartemisinin (5)	250	250

[a] Of Indochina origin, chloroquine-resistant, mefloquine-sensitive. [b] Of Sierra Leone origin, chloroquine-sensitive, mefloquine-resistant.

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Table II. Antimalarial Activity of Deoxoartemisinin against *P. berghei* in mice

compound	dosage, mg/kg	no. of mice cured
control	0	0/5
artemisinin (1)	640 × 1	5/5
	320 × 1	0/5
	160 × 1	0/5
	80 × 1	0/5
	40 × 1	0/5
	20 × 1	0/5
deoxoartemisinin (2)	640 × 1	5/5
	320 × 1	2/5, 2/5 (A)[a]
	160 × 1	0/5, 2/5 (A)[a]
	80 × 1	0/5
	40 × 1	0/5
	20 × 1	0/5

[a] A = active. The terms cure and active are defined in the Experimental Section.

temisinin without affecting the rest of the molecule was developed. Direct one-step reduction of the carbonyl function of 1 into (+)-deoxoartemisinin (2) was successfully achieved by $NaBH_4$ in the presence of $BF_3 \cdot Et_2O$ in dry THF in a yield of 71%. The biologically important endoperoxide is left intact under this reduction condition. Further hydrogenation[15] of 2 with 5% Pd/$CaCO_3$ and subsequent in situ treatment with *p*-TsOH/toluene afforded (−)-deoxodeoxyartemisinin (**5**) in 98% yield. Compound **5** was also synthesized from artemisinic acid (**6**) in four steps (overall yield 22%) (Scheme I). Thus stereoselective[7] and double reduction of **7** with $LiAlH_4$ in the presence of $NiCl_2$ gave dihydro alcohol **8**[16] (yield 51%). Ozonolysis of **8** and in situ cyclization with *p*-TsOH afforded **9** as an oil (yield 47%). Final epoxidative cyclization of **9** with *m*-CPBA in $CHCl_3$ afforded **5** cleanly and of natural configuration (93%). Deoxodeoxyartemisinin (**5**) from artemisinin (**1**) is identical by comparison of mixed melting point, specific rotation, and spectral properties with **5** from artemisinic acid (**6**). No NOE enhancement was observed between 5-H (δ 5.19, s) and 11-H (δ 2.64, m) of **2** and 5-H (δ 5.25, s) and 11-H (δ 2.27, m) of **5**, establishing that the 11-H protons of **2** and **5** are α. Thus all chiral centers of **1** and **6** were retained in their natural configuration during these manipulations.

Biological Evaluation

Table I shows in vitro assessment of the antimalarial activity of artemisinin-related drugs. Although dihydroartemisinin possesses the highest antimalarial activity reported in Table I, dihydroartemisinin is chemically unstable and thus not practical as a drug substance. (+)-Deoxoartemisinin **2** shows approximately 8 times the antimalarial activity in vitro against chloroquine-resistant malaria as compared to that of artemisinin and **2** is slightly more active than β-arteether. This result therefore suggests that an external C–O bond at C-12 either in the form of a carbonyl or a *C*-alkoxy group is not necessary for antimalarial activity. (+)-Deoxoartemisinin lacks the carbonyl function at C-12 and is projected to possess increased stability and thus longer half-life in the body. Compound **2** is crystalline and surprisingly stable: the material is recovered unchanged after heating neat for 5 min at 140 °C. (−)-Deoxodeoxyartemisinin (**5**) did not show noteworthy antimalarial activity (Table I). However, (−)-deoxodeoxyartemisinin (**5**) is a potential metabolite of deoxoartemisinin **2**. Its preparation provides the material for toxicology evaluation as well as a standard for metabolism studies. Deoxoartemisinin **2** was subjected to further testing in mice (see Table II). Of the five *Plasmodium berghei* infected mice that received 640 mg/kg in a single dose of either **2** or **1**, all five were cured. However, only two of five mice that received 320 mg/kg in a single dose of **2** were cured, while all of five mice that received the same dose of **1** were dead. Therefore **2** possesses superior in vivo antimalarial activity to **1**.

In conclusion, (+)-deoxoartemisinin, a new and more active antimalarial agent, was successfully prepared from artemisinin in one step by using a process of reductive deoxygenation. (−)-Deoxodeoxyartemisinin, a potential metabolite of deoxoartemisinin, was prepared either from (+)-deoxoartemisinin or from artemisinic acid. (+)-Deoxoartemisinin shows eight-fold antimalarial activity in vitro against chloroquine-resistant malaria as compared to that of artemisinin. The superior in vivo antimalarial activity of **2** to **1** renders the new compound a potential candidate drug for the treatment of malaria. Its further development into a new antimalarial drug against chloroquine-resistant malaria is in progress.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra (KBr) were taken on a Perkin-Elmer 281B spectrometer. Low resolution mass spectra were obtained on a Hewlett-Packard spectrometer (Model 5985) operating with electron impact at 70 eV. NMR spectra were recorded on a Varian VXR (300 MHz) using Me_4Si as an internal standard. Carbon-13 NMR assignment were reported by chemical shift followed by the number of attached hydrogens as determined from DEPT experiments and the carbon assignment is in the parentheses. Specific rotations were recorded on a Perkin-Elmer Model 141 polarimeter. Elemental analyses were performed by the Galbraith Laboratories, Inc. (Knoxville, TN).

Preparation of (+)-Deoxoartemisinin (2) from Artemisinin (1). Under a nitrogen atmosphere, artemisinin (1; 1.0 g, 3.54 mmol) was mixed with 13.20 mL of $BF_3 \cdot Et_2O$ in dry THF (20 mL) and this solution was added dropwise (3 min) to an ice-cooled, stirred solution of 0.30 g (7.93 mmol) of $NaBH_4$ in dry THF (20 mL). The solution was kept cold for 1 h and then refluxed for 10 min. After cooling again to 0 °C, ice (120 mL) was added and the mixture was extracted with Et_2O (5 × 300 mL). The extract was dried over $MgSO_4$ and concentrated in vacuo to yield crude products, which were purified by flash column chromatography (silica gel) to afford 0.67 g (71%) of homogenous product. Recrystallization from petroleum either afforded colorless crystals of **2**: mp 104–105 °C; $[\alpha]^{18}_D$ = +86.25° (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 5.19 (s, 1 H, 5-CH), 3.72 (dd, *J* = 4.2, 11.7 Hz, 1 H, 12-CH), 3.44 (t, *J* = 11.7 Hz, 1 H, 12-CH), 2.64 (m, 1 H, 11-CH), 2.37 (2 dd, *J* = 4.2, 1.2 Hz, 7-CH), 1.43 (s, 3 H, 15-CH_3), 0.96 (d, *J* = 6.3 Hz, CH_3), 0.77 (d, *J* = 7.2 Hz, CH_3); C^{13} NMR (75 MHz; $CDCl_3$) 104.16 (0, C-4), 92.13 (1, C-5), 80.78 (0, C-6), 66.22 (2, C-12), 52.18 (1, C-1), 44.86 (1, C-7), 37.30 (1, C-10), 36.23 (2, C-3), 34.02 (2, C-9), 27.96 (1, C-11), 26.11 (3, C-15), 24.72 (2, C-2), 20.72 (2, C-8), 20.32 (3, C-14), 13.12 (3, C-13); IR (KBr) 2950, 2860, 1500, 1340, 1060, 875 cm^{-1}; MS (70eV) *m/z* 268 (M^+), 250 ($M^+ - H_2O$), 236 ($M^+ - O_2$), 178, 164, 137. Anal. ($C_{15}H_{24}O_4$) C, H, O.

Conversion of (+)-Deoxoartemisinin (2) to (−)-Deoxodeoxyartemisinin (5). A 24-mg portion of 5% Pd/$CaCO_3$ was added to a solution of 80 mg (0.3 mmol) of deoxoartemisinin in EtOH (2.4 mL) and the solution was hydrogenated at room temperature and atmospheric pressure for 12 h. The reaction mixture was filtered through Celite and the filtrate was concentrated in vacuo. The residue was dissolved in dry benzene (2.4 mL) and 24 mg (0.12 mmol) of *p*-TsOH was added and stirred at room temperature for 1 h. The solvent was evaporated and the residue was dissolved in Et_2O (10 mL) and washed with distilled H_2O (2 × 5 mL). The ether layer was dried over $MgSO_4$ and concentrated in vacuo to afford a crude product. Purification by flash column chromatography (silica gel) and recrystallization from CH_3CN afforded 74 mg (98%) of colorless crystals. Com-

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pound **5**: mp 97–99°C; $[\alpha]^{18}{}_D = -50.25°$ (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 5.25 (s, 1 H, 5-CH), 3.94 (dd, *J* = 6.6, 12.3 Hz, 1 H, 12-CH), 3.28 (dd, *J* = 4.5, 12.1 Hz, 1 H, 12-CH), 2.27 (m, 1 H, 11-CH), 1.53 (s, 3 H, 15-CH_3), 0.92 (d, *J* = 7.5 Hz, CH_3), 0.89 (d, *J* = 6.0 Hz, CH_3); IR (KBr) 2920, 1500, 1380, 1109, 991, 880 cm^{-1}; MS (70eV) *m/z* 252 (M^+), 237, 191, 181, 164, 149, 106. Anal. ($C_{15}H_{24}O_3$) C, H, O.

Methyl Artemisinate (7). This compound was prepared in quantitative yield from artemisinic acid (**6**) according to the known procedure.[16]

Double Reduction of 7 to 8. A solution of **7** (13.5 g, 55.5 mmol) in anhydrous Et_2O (140 mL) was added over a solution of $LiAlH_4$ (8.42 g, 222 mmol, 16 equiv) and $NiCl_2{\cdot}6H_2O$ (8.25 g, 34.7 mmol, 0.6 equiv) in anhydrous Et_2O at room temperature under a N_2 atmosphere. The reaction mixture was stirred for 1 h at room temperature. After addition of Et_2O (1500 mL), brine (10 mL) was added dropwise, and mixture was stirred 1 h at room temperature. Then anhydrous $MgSO_4$ (80 g) was poured into the above mixture and stirring was continued for 2 h. The heterogenous mixture was filtered and the solid was washed repeatedly with Et_2O until no more product was removed. Evaporation in vacuo of the solvent gave a yellow oil, which was purified by flash column chromatography (silica gel H for TLC without gypsum) (hexane/ethyl acetate = 8/2) to afford **8**, as colorless needles (5.9 g, 51%): mp 81–81.5 °C (hexane); $[\alpha]^{25}{}_D = -7.45°$ (*c* 0.55, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 5.21 (s, 1 H, 11-H), 3.74 (dd, *J* = 10.57 and 3.3 Hz, 1 H, H-12), 3.51 (dd, *J* = 10.65 and 6.21 Hz, 1 H, H-12), 2.47 (br s, 1 H, 11-H), 1.63 (s, 3 H, CH_3-15), 1.00 (d, *J* = 6.8 Hz, 3 H, CH_3-13), 0.86 (d, *J* = 6.5 Hz, 3 H, CH_3-14); IR (KBr) 3330 (OH), 2900, 1450, 1380, 1030 cm^{-1}; MS (70 eV) *m/z* 222 (M^+), 204 ($M^+ - H_2O$), 163 ($M^+ - CH_3CHCH_2OH$) (100%), 121, 107, 105, 95, 93, 91, 81, 79, 77, 67, 55.

Conversion of 8 of Cyclic Enol Ether 9. O_3 was bubbled through a cooled (dry ice/acetone bath) solution of **8** (300 mg, 1.35 mmol) in CH_2Cl_2 and MeOH (5:1) (9 mL) during 1 h. Excess O_3 was removed with a N_2 stream during 30 min and Me_2S (1.5 mL, 20 mmol) was added dropwise. The reaction mixture was stirred for 10 min at the same temperature and then 1 h at room temperature. The solvent was removed in vacuo and the oily residue was dissolved with Et_2O (60 mL). The organic solution was washed with brine (2 × 10 mL), dried over $MgSO_4$, and the solvent was evaporated in vacuo to afford a brown liquid. The above crude mixture without purification was dissolved in anhydrous benzene (20 mL) and treated with *p*-$TsOH{\cdot}H_2O$ (few crystals), and the mixture was refluxed under a N_2 atmosphere with a Dean–Stark trap during 30 min. The solvent was removed in vacuo and oily residue was dissolved in Et_2O (40 mL). Organic solution was washed with brine (2 × 10 mL) and dried over $MgSO_4$, and the solvent was evaporated to afford a brown oil. This crude mixture was purified by flash chromatography (silica gel, hexane/EtOAc, 19:1) to give the desired compound **9** (150 mg, 47%) as a yellowish oil. Compound **9**: ^1H NMR ($CDCl_3$) δ 6.16 and 6.09 (2 br s, 1 H, olefinic H), 3.60 (m, 2 H, OCH_2), 2.54 (m, 2 H, CH_2CO), 2.12 (s, 3 H, CH_3CO), 0.96 (d, *J* = 6 Hz, CH_3), 0.89 (d, *J* = 7.0 Hz, CH_3); IR (neat) 2910, 1705 (C=O), 1645, 1450, 1150, 750 cm^{-1}; MS (70eV) *m/e* 236 (M^+).

Oxidative Cyclization of 9 to (−)-Deoxodeoxyartemisinin (5). To an ice-cooled solution of the cyclic enol ether **5** (100 mg, 0.42 mmol) in dry $CHCl_3$ (4 mL) was added *m*-CPBA (109 mg, 0.63 mmol). The mixture was stirred at 0 °C for 2 h. Evaporation in vacuo and purification on flash column chromatography (silica gel, hexane/EtOAc, 4:1) gave a solid. Recrystallization from acetonitrile afforded **5** (93 mg, 93%) as colorless crystals. All spectral data of **5** including melting point and specific rotation are identical with those of deoxodeoxyartemisinin obtained from artemisinin.

Biology. In Vitro Antimalarial Studies. The intrinsic antimalarial activities of deoxoartemisinin and various control drugs were quantitatively assessed by using modifications of the semiautomated microdilution method of Desjardins.[17] Deoxoartemisinin was dissolved in DMSO and subsequently diluted in culture medium with 10% plasma. Equimolar starting concentrations were predetermined to generate well-defined concentration curves over a 64-fold range of dilutions. Microtiter plates prepared with serial dilutions of drug and parasite suspensions (at 0.5% parasitemia and 1.5% hematocrit) were incubated at 37 °C in an air-tight plexiglass box, which was flushed with 5% oxygen, 5% carbon dioxide, and 90% nitrogen. After 24 h of incubation, cultures were labeled with tritiated hypoxanthine and incubated for an additional 18–20 h prior to the harvesting of particulate matter on fiber-glass strips. Hypoxanthine incorporation in each well was determined by scintillation spectrophotometry and served as an index of specific parasite growth rates. Computer-generated concentration–response curves were analyzed by nonlinear regression, and 50% inhibitory concentrations were calculated for each drug. The antimalarial tests were conducted with cultures obtained from the Malaria Laboratory, Division of Experimental Therapeutics, WRAIR/WRAMC, Washington, DC, MD. The W-2 culture utilized in the testing was of Indochina origin, chloroquine-resistant and mefloquine-sensitive. The other culture, D-6, used in the testing and reported in Table I, is of Sierra Leone origin, chloroquine-sensitive, and mefloquine-resistant.

In Vivo Antimalarial Studies. The suppressive blood schizonticidal and curative activities of test compounds (**2**, **1**) were determined in a test where mice were infected with 5.98×10^5 *P. berghei* parasitized cells intraperitoneally on day 0. Test compounds were dissolved in peanut oil and were administered subcutaneously once commencing on day 3. The dose levels of compounds given were 640, 320, 160, 80, 40, and 20 mg/kg. Blood films were taken on day 6 and then every 7 days until day 60. Blood schizonticidal activity was determined by monitoring blood films for the appearance of parasites and for extended survival times compared to infected, untreated controls. Mice surviving 60 days were considered cured. The infected untreated control mice (negative controls) died on either day 6 or 7. Compounds were considered active when the survival time of the treated mice was greater than twice that of the control mice, i.e., 12–14 days.

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