Antimalarial Activity of New Dihydroartemisinin Derivatives. 5. Sugar Analogues[1-4]

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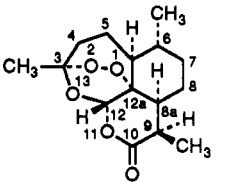
A series of dihydroartemisinin derivatives containing a sugar moiety was prepared in the search for analogues with good water solubility and high antimalarial activity. The preparation of the new compounds were achieved by treatment of dihydroartemisinin (**2**) with chlorotrimethylsilane in pyridine solution at −10 °C to give a nearly quantitative yield of 10-*O*-(trimethylsilyl)dihydroartemisinin (**3**), which was then condensed with 1-hydroxypolyacetylated sugars **5** to give dihydroartemisinin derivatives **7a–d**. Deacetylation of intermediates **7** gave the desired sugar derivatives **8**. The resulting derivatives, tested in vitro against *Plasmodium falciparum*, were found to be more effective against W-2 than D-6 clones and were not cross-resistant with existing antimalarials. Trimethylsilylated compound **3** is more effective than derivatives **7a–d**, which possess activity comparable to or better than that of artemisinin itself. Deacetylated compounds **8a–d** were substantially less active than **7** in both cell lines. In *P. berghei*-infected mice, **7a–c** showed 5/5, 2/5, and 3/5 cures, respectively, at 320 mg/kg per day × 3, whereas **7d** showed no activity at the same dosage. However, **7d** did prolong the life span in 3/5 of the infected mice at 640 mg/kg per day × 3 dose level. Trimethylsilylated compound **3** was also the most effective among the compounds studied, with 5/5 cures at 80 mg/kg per day × 3. The deacetylated sugar derivatives **8a–d** showed only slight in vivo antimalarial activity.

The prevention and treatment of malaria have been thwarted in the developing countries due to the development of drug resistance by malaria parasites to existing antimalarial agents, such as chloroquine and pyrimethamine. The search for new antimalarial drugs has become, therefore, an urgent mission of drug research programs worldwide.

Artemisinin (*qinghaosu*, **1**), a new clinically useful antimalarial agent, is an unsual sesquiterpene lactone containing an endoperoxide function.[5-9] Its unique chemical structure, coupled with its low toxicity and proven antimalarial efficacy have attracted attention from both chemists and pharmacologists since its discovery in China in the early 1970s. The practical use of artemisinin as an antimalarial agent, however, is impaired by (a) its low solubility in both water and oil,[10] (b) its poor efficacy by oral administration,[11] and (c) the high rate of recrudescence in treated patients.[11]

Extensive structure modifications of artemisinin have produced several compounds with improved solubility and efficacy. Dihydroartemisinin (**2a**), the lactol form of **1**,



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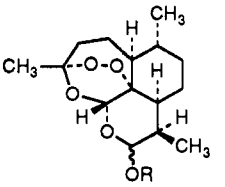

2a, R = –H
b, R = α-C(O)CH_2CH_2COOH
c, R = β-$OCH_2C_6H_4COOH$

prepared by the sodium borohydride reduction of the parent compound, was shown to be more active than **1**.[6,10] Artemether[10-13] and arteether,[10,12-16] the methyl and ethyl ethers of **2a**, respectively, are more lipophilic and also more effective than artemisinin. Sodium artesunate (**2b**), a water-soluble derivative of **2a**, was demonstrated to be particularly useful in the treatment of cerebral malaria.[10,11,15] Being the half succinic acid ester of **2a**, the compound is, however, not stable in aqueous solution,[1] to the detriment of its practical value as an antimalarial agent.

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Table I. Physical Properties of Sugar Derivatives of Dihydroartemisinin 7a–d and 8a–d

compd	mp, °C	recrystn solvent	TLC solvent	R_f	% yield	formula
7a	172–173	hexane + EtOAc	EtOAc/hexane (1:2 v/v)	0.29	48.0	$C_{29}H_{42}O_{14}$
7b	167–170	MeOH + H_2O	EtOAc/hexane (1:1 v/v)	0.69	57.1	$C_{29}H_{42}O_{14}$
7c	118–120	MeOH + H_2O	EtOAc/hexane (1:3 v/v)	0.61	57.4	$C_{27}H_{42}O_{10}$
7d	100–101	MeOH + H_2O	EtOAc/hexane (1:1 v/v)	0.27	35.0	$C_{41}H_{58}O_{22}$
8a	183–184	acetone + MeOH	5% MeOH/EtOAc	0.35	50.7	$C_{21}H_{34}O_{10}$
8b	94–96	acetone + MeOH	MeOH/EtOAc (1:5 v/v)	0.54	70.9	$C_{21}H_{34}O_{10}$[a]
8c	101–103	MeOH + EtOAc	EtOAc/hexane (2:1 v/v)	0.63	81.8	$C_{24}H_{38}O_{10}$[b]
8d	168–170	MeOH + acetone	EtOAc/hexane (4:1 v/v)	0.36	78.0	$C_{27}H_{44}O_{15}$

[a] Calcd for H = 7.68; found H = 7.24. [b] Calcd for C = 59.24; found C = 59.74.

In 1987, we reported on a series of dihydroartemisinin derivatives (**2a**) in which the solubilizing group, carboxylate, was coupled to dihydroartemisinin by an alkyl ether rather than an ester linkage.[1,2] Among the water-soluble derivatives that were prepared, the sodium salt of artelinic acid (**2c**) was found to be as active as the parent artemisinin and sodium artesunate (**2b**) in vitro and more active than **1** and **2b** in the rodent *P. berghei* test system. Furthermore, sodium artelinate is substantially more stable than sodium artesunate in weakly alkaline aqueous solution, an important chemical property necessary for the preparation of an intravenous pharmaceutical dosage form. Antimalarial studies have shown that sodium artelinate can totally eliminate the parasitemia in mice infected with *P. berghei* when administered in their drinking water, given by intubation,[16] or administered transdermally.[17]

Analogues in which a heterocyclic ring displaced the C_{10}-OH and a bromine atom substituted at C_9 of **2a** possess in vitro activity but are devoid of in vivo antimalarial activity.[3]

In continuing our search for new artemisinin analogues with enhanced water solubility and high antimalarial efficacy, we report here on the preparation and antimalarial studies of additional dihydroartemisinin derivatives which contain a sugar moiety.

Chemistry

The starting material, dihydroartemisinin (**2a**), was prepared by sodium borohydride reduction of **1** according to a modified literature procedure.[2] We have found that the use of absolute methanol containing <0.01% water as the reaction medium and adjustment of the reaction mixture to pH 5–6 before workup are critical for good yield.

Treatment of dihydroartemisinin (**2a**) with chlorotrimethylsilane in pyridine solution at −10 °C gave a nearly quantitative yield of 10-*O*-(trimethylsilyl)dihydroartemisinin (**3**), an essential intermediate for this series (Scheme I). Another intermediate **5** was prepared by regioselective 1-*O*-deacylation of the fully acetylated sugars (**4**) with sodium methoxide in THF, using the procedure of Itoh et al.[18] Treatment of **3** and **5** with a catalytic amount of trimethylsilyl trifluoromethanesulfonate[19] in CH_2Cl_2 at −78 °C under nitrogen yielded the acetylated sugar–dihydroartemisinin derivatives **7** (Table I).

The condensation of **3** and **5** created two unknown chiral centers at C_{10} and $C_{1'}$ of the product **7**. The NMR data

Scheme I

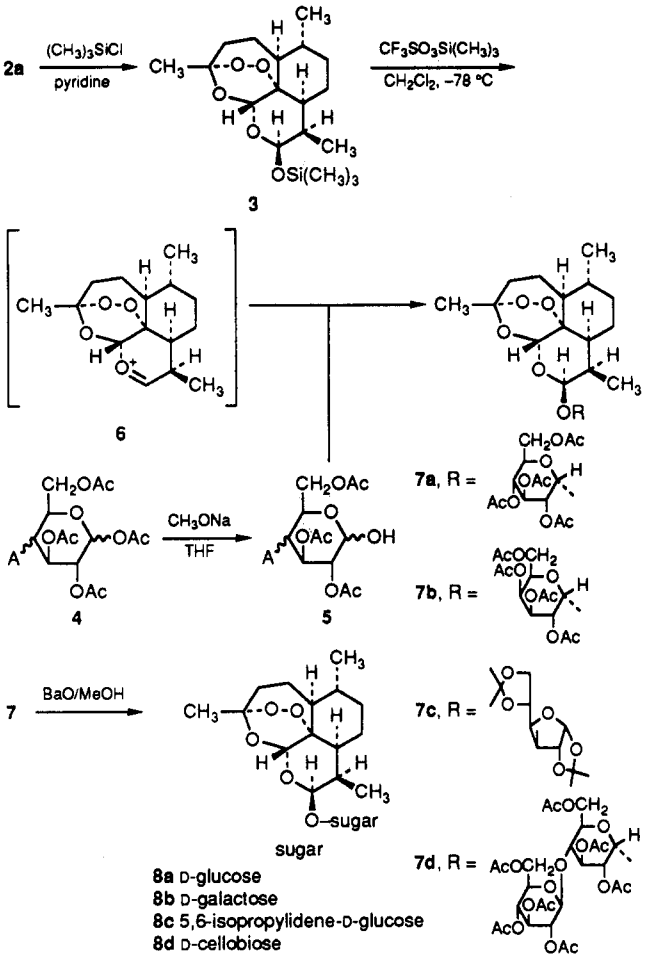

Table II. IR and NMR Data of Compounds 7a–d

compd	IR (KBr, cm^{-1})	^1H NMR (δ)
7a	1758	0.94 (d, 3 H, J = 8.0 Hz), 1.41 (s, 3 H), 2.01 (s, 3 H), 2.04 (s, 3 H), 2.08 (s, 3 H), 2.17 (s, 3 H), 4.0–5.48 (m, 9 H)
7b	1758	0.96 (d, 3 H, J = 7.2 Hz), 1.41 (s, 3 H), 1.60 (s, 3 H), 1.99 (s, 3 H), 2.04 (s, 3 H), 2.09 (s, 3 H), 2.16 (s, 3 H), 4.04–4.26 (m, 6 H), 5.06 (d, 1 H, J = 2.7 Hz), 5.40 (s, 1 H), 5.30 (d, 1 H, J = 3.6 Hz)
7c		0.97 (d, 3 H, J = 6.3 Hz), 1.33 (d, 3 H, J = 2.7 Hz), 1.40 (s, 3 H), 1.45 (s, 3 H), 1.49 (s, 3 H), 1.50 (s, 3 H), 1.60 (s, 3 H), 2.17 (s, 3 H), 4.10 (m, 3 H), 4.30 (d, 1 H, J = 1.8 Hz), 4.63 (d, 1 H, J = 3.6 Hz), 5.11 (d, 1 H, J = 3.6 Hz), 5.39 (s, 1 H), 5.85 (d, 1 H, J = 3.6 Hz)
7d	1756	0.98 (d, 3 H, J = 6.3 Hz), 1.39 (s, 3 H), 1.58 (s, 3 H), 3.72–5.49 (m, 16 H)

(Table II) shows the coupling constant between C_9-H and C_{10}-H of **7** to be J = 2.7 Hz, indicating the absolute con-

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Table III. In Vitro Antimalarial Activities of Dihydroartemisinin Derivatives against *P. falciparum*

compd	50% inhibitory concentration (IC_{50}, ng/mL)	
	Sierra Leone clone (D-6)	Indochina clone (W-2)
1	2.94	0.68
3	1.11	0.42
7a	2.48	0.19
7b	3.00	1.06
7c	1.43	0.31
7d	5.78	2.73
8a	121.5	34.07
8b	13.13	35.60
8c	2.57	0.82
8d	710	171

figuration at C_{10} to be β. On the other hand, the coupling between C_1-H and C_2-H of **7** is $J = 3.2$ Hz, indicating the C_1-H and C_2-H are cis and thus the absolute configuration at $C_{1'}$ is α. The relationship between coupling constant and configuration of glycosides and ether derivatives of dihydroartemisinin are well documented.[1-5,14,20-22]

The most likely mechanism of reaction between **3** and **5** involves the formation of reactive oxonium **6** as the reactive intermediate which reacts with the hydroxy function of **5** to give the observed product **7**. The chiral centers in **6** effect the preferential approach of **5** from β-side of **6**. A similar mechanism was reported for the stereospecific formation of ether or amino derivatives of dihydroartemisinin.[1-4]

The deprotection of the acetylated sugar–dihydroartemisinin conjugates **7** was carried out with barium oxide in methanol to give the desired dihydroartemisinin sugar derivatives **8** in 50–85% yield (Table I). The protecting group, 2,3-*O*-isopropylidene, in **7c** was removed by hydrolysis in dilute acetic acid at room temperature. Under the same conditions, however, it failed to deprotect the 5,6-isopropylidene group of **7c**.

Results and Discussion

The new derivatives were tested for inhibitory properties in vitro against two clones of human malaria *P. falciparum* D-6 (Sierra Leone clone), which is resistant to mefloquine and W-2 (Indochina clone), which is resistant to chloroquine, pyrimethamine, sulfadoxine, and quinine.

The results (Table III) indicate that the new derivatives, like the parent compound **1**,[1-3] are not cross-resistant with any of the existing antimalarial agents mentioned above. Similar to the other ether derivatives of this class, the compounds are, in general, more effective against the W-2 than the D-6 strain. The acetylated sugar–dihydroartemisinin derivatives **7** possess activity comparable to or better than that of the parent molecule, artemisinin (**1**). However, deacetylation of **7** gave the final glycoside products **8** which were substantially less active than **7** in both cell lines. It is interesting to note that the intermediate, trimethylsilylated compound **3**, is more effective than the derivatives **7a–d**.

The new compounds were also tested against *P. berghei* in mice (Table IV). While the acetylated compounds **7a–d** show moderate in vivo activity, the deacetylated or deprotected compounds **8a–d** are inactive in the in vivo test system. Among the active compounds, **7a** shows 5/5 cures in infected mice at dose of 320 mg/kg per day for 3 days, whereas **7b** and **7c** showed 2/5 and 3/5 cures, respectively, at the same dose level. Compound **7d** (a diglycoside) shows no antimalarial activity up to 320 mg/kg; however, at a higher dosage (640 mg × 3), **7d** increased survival time for 3/5 mice. As indicated earlier, the trimethylsilylated compound **3** is the most active among all the compounds tested in this study, giving 5/5 cures at 80 mg/kg per day × 3. The antimalarial results indicated that the in vitro activity of the new compounds parallel those observed in vivo tests and that the increase in polarity or water solubility tends to decrease antimalarial activity.

Table IV. Antimalarial Activity of Dihydroartemisinin Derivatives against *P. berghei* in Mice

compd	dosage, mg/kg per day[a]	no. of mice cured[b]
control		0/5
3	320	4/5
	80	5/5
	20	1/5, 4/5 (A)
7a	320	5/5
	80	0/5, 4/5 (A)
	20	0/5
7b	640	5/5
	320	2/5, 1/5 (A)
	160	0/5, 2/5 (A)
	80	2/5, 1/5 (A)
	40	0/5
7c	640	4/5, 1/5 (A)
	320	3/5, 2/5 (A)
	160	2/5, 3/5 (A)
	80	0/5, 4/5 (A)
	40	0/5
7d	640	0/5, 3/5 (A)
	320	0/5
	160	0/5
	80	0/5
	40	0/5

[a] Once daily for 3 consecutive days. [b] The term cure and active (A) are defined in the Experimental Section.

Experimental Section

Chemistry. All melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra of solid samples were obtained in KBr disks on a Nicolet 20SXB FT-IR spectrometer. NMR spectra were determined on a JEOL FX90Q spectrometer with Me_4Si as an internal standard. Elemental analyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, MI, and the results were within 0.4% of the theoretical values, except where noted.

Preparation of 10-*O*-(Trimethylsilyl)dihydroartemisinin (3). To a solution of dihydroartemisinin[1,2] (**2a**, 1.5 g, 5.28 mmol) in 35 mL of anhydrous pyridine was added dropwise chlorotrimethylsilane (5 mL, 39.4 mmol) at −10 °C. The solution was stirred at room temperature for 24 h, poured into 100 mL of ice water, extracted twice with 100 mL of CH_2Cl_2, washed with H_2O, dried over $MgSO_4$, and evaporated to dryness under the reduced pressure. The crude product was subjected to silica gel column chromatography, using hexane/EtOAc (20:1 v/v) as eluent to give 1.8 g (93%) of the product as white crystals. Recrystallization from MeOH and H_2O gave white crystals, mp 40–41 °C. Anal. ($C_{18}H_{32}O_5Si \cdot 0.5H_2O$) C, H.

Regioselective 1-O-Deacylation of Fully Acylated Sugars (5). To a suspension of 97% sodium methoxide (3.0 g, 5.4 mmol) in 50 mL of THF which was cooled in an ice–salt bath was added 20.5 mmol of the acetylated sugar α-D-glucose pentaacetate. The mixture was stirred for 6 h, quenched with 3 mL of acetic acid, and evaporated to dryness under the reduced pressure. The crude product was dissolved in CH_2Cl_2, washed with water three times, dried over $MgSO_4$, and evaporated to dryness. The residue was purified by silica gel flash column chromatography using C_6H_6/EtOAc (1:1 v/v) as solvent to give 5 g (70%) of the desired regioselective 1-O-deacylated sugar 1-hydroxy-2,3,4,6-tetra-*O*-

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acetyl-D-glucose as a gum, which was used for next reaction without further purification.

The regioselective 1-O-deacylations of other acylated sugars were performed similarly. Their corresponding 1-hydroxy sugar derivatives are 1-hydroxy-2,3,4,6-tetra-*O*-acetyl-D-galactose, 3-hydroxy-1,2:5,6-diisopropylidene-D-glucose, and 1-hydroxy-2,3,6-tri-*O*-acetyl-4-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranose.

General Procedure for the Condensation of 10-*O*-(Trimethylsilyl)dihydroartemisinin (3) and 1-OH-Acetylated Sugars (5). One millimole of 10-*O*-(trimethylsilyl)dihydroartemisinin (3) and 1 mmol of a 1-OH-acetylated sugar (5) were dissolved in 10 mL of dry CH_2Cl_2. To the solution was added a catalytic amount of trimethylsilyl trifluoromethanesulfonate (0.1 mL in 2 mL of dried CH_2Cl_2). The mixture was stirred at −78 °C under N_2 for 5 h, and the reaction was quenched by the addition of 0.5 mL of triethylamine. The solution was diluted with 50 mL of CH_2Cl_2, washed successively with saturated $NaHCO_3$ and H_2O, dried over $MgSO_4$, and evaporated to dryness under the reduced pressure. The residue was purified on a silica gel column (Table I).

General Procedure for the Deacylation of the Acetylated Artemisinin–Sugar Derivatives. To a solution of 7 (1 mmol) in 15 mL of MeOH was added 0.5 mL of BaO (0.38 g of BaO in 15 mL of MeOH). The solution was cooled in an ice bath. After stirring for 4.5 h, the solution was acidified with dilute HOAc to pH 5–6 and evaporated under reduced pressure. The residue was subjected to silica gel column chromatography separation to give the desired product. The physical properties of the sugar derivatives are listed in Table I.

Biology. (a) In Vitro Antimalarial Studies. The in vitro assays were conducted by using a modification of the semiautomated microdilution technique of Desjardins et al.[23] and Milhous et al.[24] Two *P. falciparum* malaria parasite clones, designated as Indochina (W-2) and Sierra Leone (D-6), were utilized in susceptibility testing. They were derived by direct visualization and micromanipulation from patient isolates obtained by the Centers for Disease Control, Atlanta, GA, in 1980 and 1982, respectively. The patients had acquired infections either in Vietnam or in Sierra Leone. The Indochina clone is resistant to the antimalarials, chloroquine, sulfadoxine, pyrimethamine, and quinine, whereas the Sierra Leone clone is resistant to mefloquine but susceptible to chloroquine, quinine, sulfadoxine, and pyrimethamine. Test compounds were initially dissolved in DMSO and 70% ethanol and diluted in RPMI 1640 culture medium with 10% human plasma to 400-fold. Drugs were subsequently further diluted by using the Cetus Pro/Pette (Perkin-Elmer Corp., Norwalk, CT) over a range of $(1.56–100) \times 10^{-9}$ M. Parasite inocula (at 0.5% parasitemia and a 1% hematocrit) were incubated for 24 h and added to equimolar concentrations of each test compound prior to the addition of [^{3}H]hypoxanthine. After a further incubation of 18 h, parasitized red blood cells were harvested from each microtiter well by using an automated cell harvester (Skatron Inc., Sterling, VA). Uptake of [^{3}H]hypoxanthine was measured by using a scintillation spectrophotometer (Model LS3801, Beckman Instruments, Irvine, CA). Concentration–response data were analyzed by nonlinear regression, and the IC_{50} values (50% inhibitory concentrations) for each compound were calculated.

(b) In Vivo Antimalarial Studies. The suppressive blood schizonticidal and the curative activities of the compounds were measured in a test in which mice were infected with 5.89×10^5 *P. berghei* parasitized cells intraperitoneally on day 0. Test compounds were dissolved in either peanut oil or 5% $NaHCO_3$ aqueous solution and were administered subcutaneously once a day for 3 consecutive days commencing on day 3. The dose levels of compounds given were 640, 320, 160, 80, and 40 mg/kg per day. Blood films were taken on days 6, 13, and 20. 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 was considered active when the survival time of the treated mice was greater than twice the control mice, i.e., 12–14 days.

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