Antimalarial Activity of New Dihydroartemisinin Derivatives. 6. α-Alkylbenzylic Ethers[1–5]

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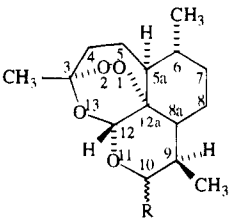
A series of diastereomeric dihydroartemisinin α-alkylbenzylic ethers was synthesized in search for analogs with higher antimalarial efficacy and longer plasma half-life than the existing artemisinin derivatives. Artelinic acid was used as the model molecule for the design of new analogs. Two approaches were taken in an attempt to (a) increase the lipophilicity of the molecule and (b) decrease the rate of oxidative dealkylation of the target compounds. All compounds in this study showed at least equal or better *in vitro* antimalarial activity against *Plasmodium falciparum* than artelinic acid. The most active compounds of this series showed 10-, 20-, and 40-fold better inhibitory activity than artemether, artemisinin, and artelinic acid, respectively. Compounds which have a small methyl group substituted at the α-methylene group showed weaker activity than compounds with a larger carbethoxyalkyl substituent, indicating that the lipophilicity and the steric effect of the molecules play important roles in their antimalarial activity. This fact is further substantiated by the significantly weaker antimalarial activity of the carboxylic acids than their corresponding esters. Compounds with electron-withdrawing function (NO_2) substantially increase the antimalarial activity. The *S*-diastereomers, in general, are severalfold more potent than the corresponding *R*-isomer.

Background

The effectiveness of artemisinin (**1a**) and its derivatives as antimalarial drugs for the treatment of chloroquine, mefloquine, or multidrug resistant *Plasmodium falciparum* has received increasing attention in recent years.[6–10] The practical value of artemisinin, however, is impaired by its (a) high rate of recrudescence,[8] (b) poor solubility in oil and water,[7] (c) short plasma half-life,[11,12] and (d) poor oral activity (active only at very high dosage).[8] Chemical modifications of artemisinin have resulted in a number of analogs with improved efficacy and increased solubility in either oil, i.e., arteether and artemether[7,13] (**1b**,**c**), or water, i.e., sodium artelinate[14] (**1d**) and sodium artesunate[15] (**1e**). Both artemether and arteether showed higher potency than artemisinin but have short plasma half-lives and severe central nervous system (CNS) toxicity in rats and dogs.[16,17] Likewise, the usefulness of sodium artesunate in the treatment of cerebral malaria and multidrug resistant *P. falciparum* was offset by problems associated with its lability to chemical hydrolysis in aqueous solution,[1] the high rate of recrudescence, and the extremely short plasma half-life (20–30 min).[16a]

Sodium artelinate was designed to overcome the instability of sodium artesunate in aqueous solution. It was found that sodium artelinate is not only very stable in aqueous solution but also has a much longer plasma half-life (1.5–3 h) than artemether, arteether, and sodium artesunate.[1,16] Its antimalarial activity against *Plasmodium berghei in vivo* is comparable to or slightly better than that of artemisinin and sodium artesunate by subcutaneous injection. However, it is highly effective when administered orally at 240 mg/kg total dose[18] and showed complete cure against *Plasmodium knowlesi* infection in rhesus monkeys, a model test system for cerebral malaria, at 15 mg/kg × 3 days.[19] Therefore, sodium artelinate has been considered the best candidate drug among the available water soluble analogs of this class for the treatment of comatose cerebral malaria. Furthermore, recent CNS toxicity studies indicated that the water soluble dihydroartemisinin derivatives such as artesunate and artelinate possess substantially less CNS toxicity in rat and dog than oil soluble analogs such as artemether and arteether.[17]



OIL SOLUBLE

1a R = =O
ARTEMISININ

1b R = OCH_2CH_3
β–ARTEETHER

1c R = OCH_3
β– ARTEMETHER

WATER SOLUBLE

1d R = OCH_2–⟨◯⟩–$COONa$
β-SODIUM ARTELINATE

1e R = $OCOCH_2CH_2COONa$
α-SODIUM ARTESUNATE

Pharmacokinetic data of arteether, artemether, and artesunic acid demonstrated a rapid conversion of these compounds to dihydroartemisinin (DQHS) *in vivo* or in liver homogenate.[16] Since the ether linkage is rather inert to chemical hydrolysis, the *in vivo* transformation of dihydroartemisinin derivatives to DQHS must be an enzymatic rather than a chemical reaction. Our previous studies on the thermolysis products of DQHS and the reported chemical hydrolysis products of artemisinin strongly indicate the involvement of a hydroperoxide-containing intermediate for the isolated rearrangement or decomposition products.[20] On the basis of this chemical information, it is likely that dihydroartemisinin (**3**), once formed enzymatically, will undergo chemical rearrangement to generate hydroperoxide **4** (Scheme

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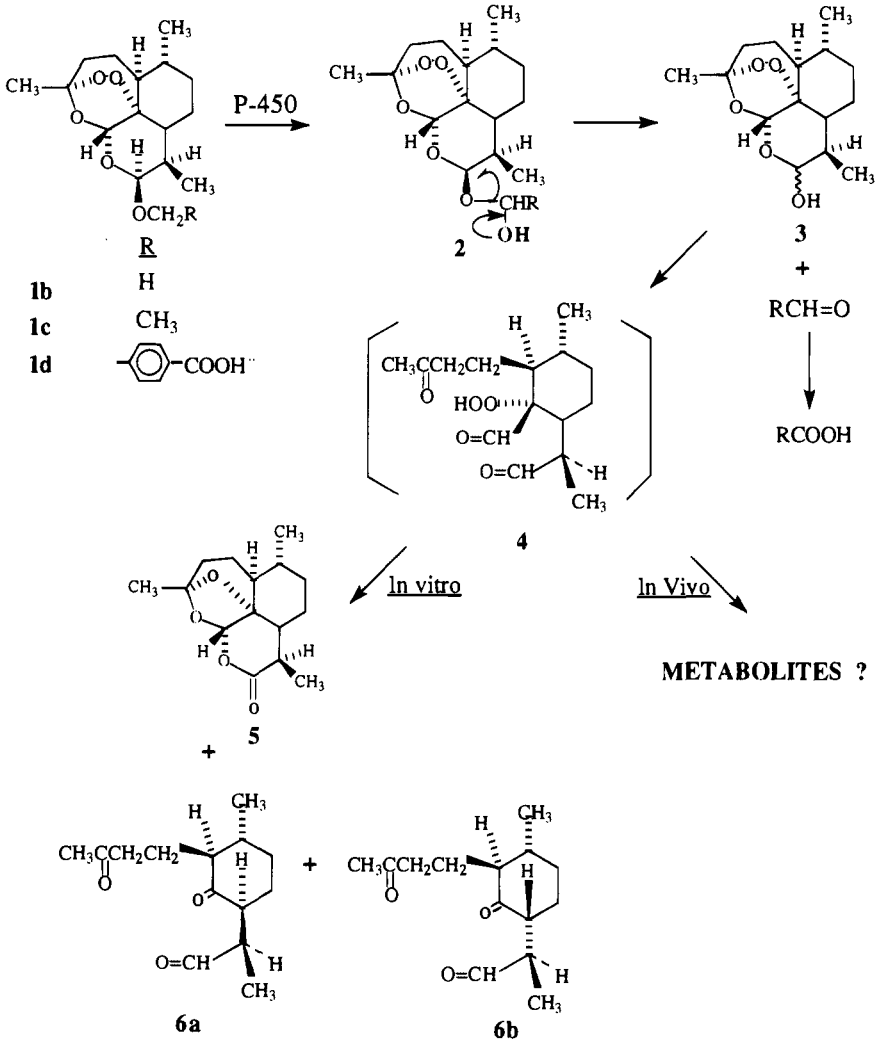
Scheme 1. Biochemical and Chemical Transformations of Dihydroartemisinin Derivatives

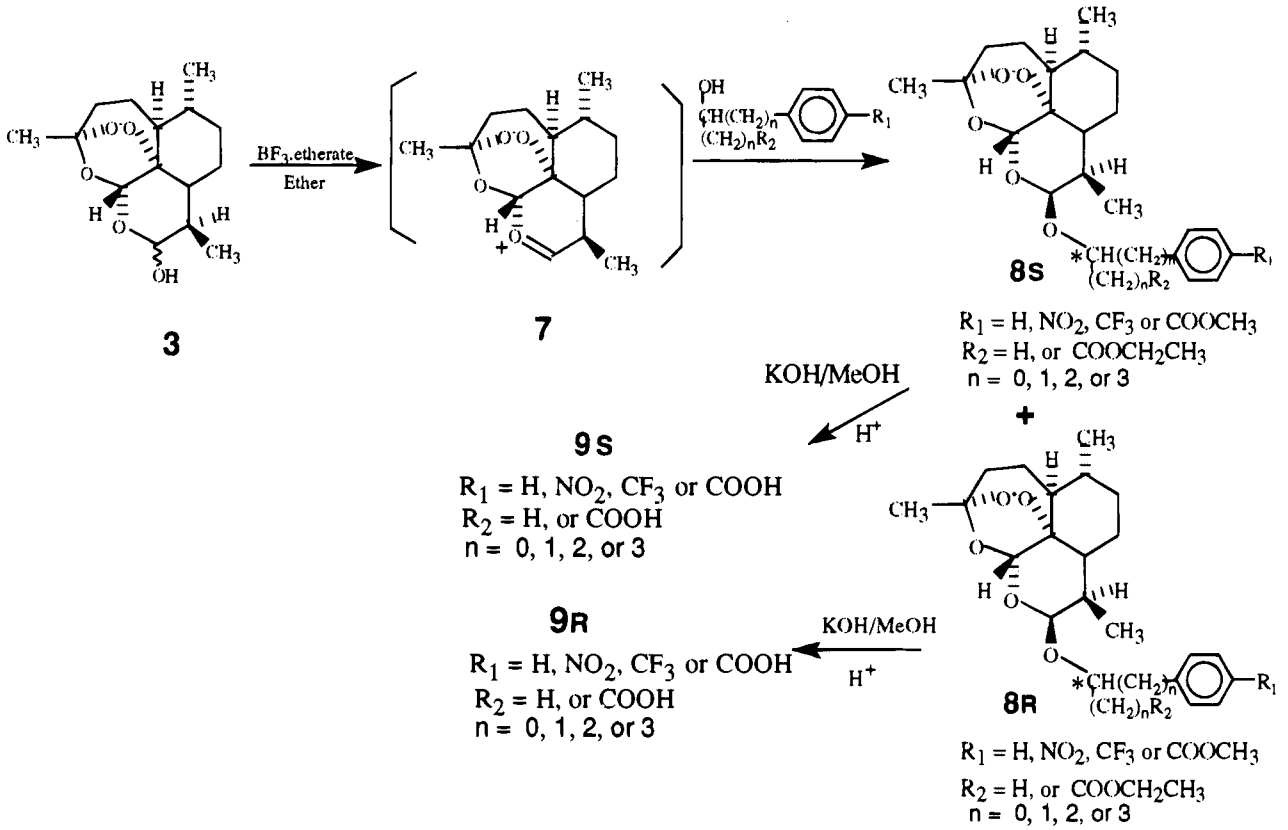


1). The intermediate **4** contains hydroperoxide, aldehyde, and ketone groups which are known to be reactive and toxic and thus may be responsible for the observed parasite cell kill. This view is substantiated by the fact that the deoxydihydroartemisinin, which lacks a peroxide and is unable to generate hydroperoxide, is an inactive compound. Since DQHS is a highly potent antimalarial compound itself and most of the known derivatives of DQHS were found to convert to DQHS *in vivo*, derivatives of DQHS, such as artemether, arteether, and artesunate, are considered prodrugs of dihydroartemisinin.

The high rate of recrudescence and the low rate of radical cure of dihydroartemisinin derivatives in general may be, at least in part, due to the short plasma half-life of the compounds, and the short plasma half-life may be a result of fast conversion of the prodrug to DQHS. The conversion of artemether, arteether, and artelinic acid to DQHS has been demonstrated to involve an enzymatic hydroxylation by cytochrome P-450 to generate a hemiacetal intermediate, **2**, which decomposed to produce DQHS and aldehyde (Scheme 1).[16a,b] Plasma half-life of these derivatives, therefore, depends on the rate of oxidative dealkylation (in the case of arteether or artemether) or deacylation (in the case of artesunate) to generate the active component, DQHS. The fact that the artelinic acid has a longer plasma half-life than artemether or arteether may be due to its slower rate of dealkylation to DQHS than artemether and arteether. Therefore, the feasible approach to prolong the plasma half-life of this class of compounds is to fabricate new analogs that are a poorer substrate for cytochrome P-450 than artelinic acid (**1d**). This can be achieved by (a) manipulating the electron density of the aromatic ring of artelinic acid by introduction of either an electron-donating or an electron-withdrawing group or (b) exerting steric effect to the α-methylene carbon (hydroxylation site) by introduction of a substituent at the α-methylene carbon or at the benzene ring *ortho* to the methylene group. The inductive effects of the substituted aromatic ring will thus affect the electron density of the methylene function and consequently alter its substrate properties. The steric effect of the substituents at the *ortho* position should interfer with the rate of hydroxylation of the α-methylene group and thus should prolong the plasma half-life and enhance the efficacy of the molecule. In as much as the rate of hydroxylative dealkylation of the DQHS derivatives may determine their plasma half-life and thus their efficacy, substituents for optimal potency can be achieved by systematic chemical modifications.

As part of an ongoing research project in search for stable, water soluble, high-potency, long acting, and orally active antimalarial drugs with minimum CNS toxicity, a series of new dihydroartemisinin α-alkylbenzylic ethers, based on the above-mentioned rationale,

Scheme 2



were synthesized and their *in vitro* antimalarial activity was assessed against two clones (D-6 and W-2) of *P. falciparum*.

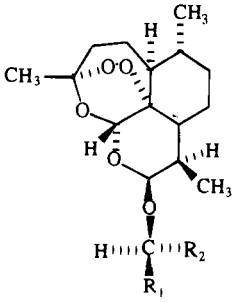
Chemistry

Dihydroartemisinin (**3**) was prepared by sodium borohydride reduction of artemisinin as previously reported.[1,2] The new ether derivatives **8** of dihydroartemisinin were prepared by treatment of **3** with an appropriate alcohol in the presence of boron trifluoride etherate at room temperature (Scheme 2). The yield of the purified condensation products **8** ranged from 40 to 90% (Table 1). The purification was achieved by the use of a silica gel chromatotron, preparative TLC, or column chromatography. In previous work related to this study,[1-5] the condensation between dihydroartemisinin and benzylic alcohols gave the β-isomer as the major condensation product which has small coupling constants (J = 3.5 Hz) between C_9-H and C_{10}-H. The α-isomer, which has a larger coupling constant (J = 9–10 Hz) between C_9-H and C_{10}-H than the β-isomer, was less than 10% of the condensation products. The relationship between coupling constant and the configuration at the C_{10} position was discussed in previous papers of this series.[1,2] An oxonium ion, **7**, was proposed to be involved in the ether formation.[1,2] Likewise, the condensation reaction between dihydroartemisinin (**3**) and the racemic mixture of α-substituted benzylic alcohols gave predominantly the β-isomers. Some of the starting alcohols are commercially available. Those which are not available from commercial sources were prepared by esterification of the corresponding keto acids in boiling absolute methanol in the presence of boron trifluoride etherate, followed by sodium-borohydride reduction of the keto ester to give the racemic alcohols.

Although the resolution of a racemic mixture to single R- and S-isomers is difficult and tedious, the multiasymmetric carbon centers of the dihydroartemisinin fused ring moiety serves as an excellent internal resolving agent for its diastereomeric derivatives whose two new chiral centers were created during the derivatization. The dihydroartemisinin moiety, therefore, renders the new diastereomeric derivatives dissimilarity in their physical properties such as melting point, solubility, and R_f values in various solvent systems. Therefore, the diastereomers of compounds with an asymmetric carbon center on the ether side chain (**8**) were successfully separated by the use of a normal phase silica gel chromatron, preparative TLC, or a silica gel column using ethyl acetate and hexane or petroleum ether as eluent. The hydrolysis of **8** with 2.5% KOH/MeOH gave the corresponding carboxylic potassium salt which was converted to the free carboxylic acids by the addition of dilute HCl to give **9**. Although saponification of **8j,k** gave good yield of **9a,b**, the yield of **9c** under the same conditions is very poor (less than 5%). This is due to the predominant β-elimination of the dihydroartemisininoxy group from **8h** during the saponification process, as evidenced by the isolation and identification of ethyl 3-(4-nitrophenyl)acrylate and its corresponding acid. The identity of all products were established by ^{1}H-NMR (Table 2) spectrometry and elemental analysis.

The proton NMR spectra of R- and S-diastereomers of **8** and **9** display large chemical shift differences (Δ ppm) from signals of C_{10}-H and C_{12}-H of the dihydroartemisinin moiety. The proton signals of C_{10}-H from R-isomers of this class resonate 0.03–0.54 ppm upfield from that of the corresponding S-isomers (Figure 1) with the exception of **8c,d**. On the contrary, the proton signals from C_{12}-H of R-isomers are 0.35–1.04 ppm downfield from that of S-isomers, again, with the

Table 1. Physical Properties of the Esters **8** and the Carboxylic Acids **9**



compd	R_1	R_2	$[\alpha]^{23}_D$[a]	mp (°C)	formula	TLC solvent	R_f	yield (%)
8a (*R*)	$-CH_2CH_2CH_3$	$-CH_2-C_6H_5$	+130	124.7	$C_{26}H_{38}O_5$	1.5% EtOAc/$CHCl_3$	0.76	20
8b (*S*)	$-CH_2-C_6H_5$	$-CH_2CH_2CH_3$	+161	148.7	$C_{26}H_{38}O_5$	1.5% EtOAc/$CHCl_3$	0.68	53
8c (*R*)	$-C_6H_5$	$-COOCH_2CH_3$	+220	gum	$C_{25}H_{34}O_7 \cdot {}^2/_3H_2O$	EtOAc/hexane, 1:4	0.53	51
8d (*S*)	$-COOCH_2CH_3$	$-C_6H_5$	+177	84.9	$C_{25}H_{34}O_7 \cdot H_2O$	EtOAc/hexane, 1:4	0.58	38
8e (*R*)	$-CH_3$	$-C_6H_4-CF_3$	+187	126	$C_{24}H_{31}O_5F_3$	EtOAc/petroleum ether, 1:5	0.82	60
8f (*R*)	$-CH_2COOCH_2CH_3$	$-C_6H_5$	+197	95.2	$C_{26}H_{36}O_7 \cdot {}^1/_4H_2O$	EtOAc/petroleum ether, 1:6	0.77	30
8g (*S*)	$-C_6H_5$	$-CH_2COOCH_2CH_3$	+94	gum	$C_{26}H_{36}O_7$	EtOAc/petroleum ether, 1:6	0.62	6
8h (*R*)	$-CH_2COOCH_2CH_3$	$-C_6H_4-NO_2$	+150	116.3	$C_{26}H_{35}NO_9$	EtOAc/hexane, 1:3	0.52	28
8i (*S*)	$-C_6H_4-NO_2$	$-CH_2COOCH_2CH_3$	+67	131.2	$C_{26}H_{35}NO_9$	EtOAc/hexane, 1:3	0.45	28
8j (*R*)	$-CH_3$	$-C_6H_4-COOCH_3$	+164	72	$C_{25}H_{34}O_7$	EtOAc/petroleum ether, 1:6	0.58	43
8k (*S*)	$-C_6H_4-COOCH_3$	$-CH_3$	+123	gum	$C_{25}H_{34}O_7$	EtOAc/petroleum ether, 1:6	0.49	36
9a (*R*)	$-CH_3$	$-C_6H_4-COOH$	+108	97.2	$C_{24}H_{32}O_7$	recrystd from EtOAc/petroleum ether		40
9b (*S*)	$-C_6H_4-COOH$	$-CH_3$	+44	82.2	$C_{24}H_{32}O_7 \cdot H_2O$	recrystd from EtOAc/petroleum ether		42
9c (*R*)	$-CH_2COOH$	$-C_6H_4-NO_2$	+170	156.5	$C_{24}H_{31}NO_9$	recryst EtOAc + petroleum ether		11

[a] [α] in 0.1% chloroform solution.

Table 2. Chemical Shifts (δ) of Compounds **8** and **9**

compd	C_9-CH_3 (d, Hz)	C_{10}-H (d, Hz)	C_{12}-H (s)	−OCH-Ar
8a (*R*)	0.76 (7.3)	4.87 (3.4)	5.44	4.0 (m)
8b (*S*)	0.85 (7.2)	4.90 (3.5)	4.41	4.13 (m)
8c (*R*)	1.13 (7.3)	5.04 (3.5)	5.48	5.26 (s)
8d (*S*)	0.89 (7.3)	4.82 (3.5)	5.63	5.22 (s)
8e (*R*)	0.85 (7.3)	4.63 (3.4)	5.52	5.03 (q, J = 6.5 Hz)
8e (*S*)	1.00 (7.3)	5.09 (3.6)	5.19	5.01 (q, J = 6.4 Hz)
8f (*R*)	0.83 (7.3)	4.60 (3.5)	5.57	5.36 (dd, J = 4, 10 Hz)
8g (*S*)	0.91 (7.5)	5.08 (3.5)	4.76	5.12 (dd, J = 5, 9 Hz)
8h (*R*)	0.88 (7.4)	4.56 (3.5)	5.59	5.50 (dd, J = 4, 10 Hz)
8i (*S*)	0.93 (7.3)	5.10 (3.5)	4.79	5.22 (dd, J = 6, 9 Hz)
8j (*R*)	0.84 (7.3)	4.64 (3.5)	5.52	5.02 (q, J = 6.6 Hz)
8k (*S*)	0.99 (7.3)	5.07 (3.6)	5.17	4.99 (q, J = 6.3 Hz)
9a (*R*)	0.86 (7.3)	4.66 (3.4)	5.53	5.05 (q, J = 6.6 Hz)
9b (*S*)	1.00 (7.3)	5.10 (3.5)	5.19	5.02 (q, J = 6.4 Hz)
9c (*R*)	0.88 (7.3)	4.57 (3.4)	5.59	5.49 (dd, J = 4, 9 Hz)

exception of **8c,d** whose signals from C_{12}-H of *R*-isomer resonate 0.15 ppm upfield to that of *S*-isomer. The substantial differences in chemical shifts observed between *R*- and *S*-isomers were found, by X-ray crystallographic data, to be due to the conformational differences in the two diastereomers.[21] The X-ray crystallography of **8j** (*S*) and **9c** (*R*) indicated that the aromatic ring of the *R*-isomer is positioned over C_{10}-H and away from C_{12}-H. On the contrary, the benzene ring of the *S*-isomer is positioned over C_{12}-H and away from C_{10}-H. The diamagnetic anisotropic effects of the benzene ring, therefore, shielded C_{10}-H of the *R*-isomer and C_{12}-H of the *S*-isomer, respectively, and thus are responsible for the observed differences in the chemical shifts. It is to be noted that the determination of *R*- and *S*-configuration is based on the conventional priority assignment of the substituents at the chiral center. According to the rules of *R,S*-configurational nomenclature, the $COOCH_2CH_3$ group of **8c,d** has higher priority than the benzene ring, while in the other

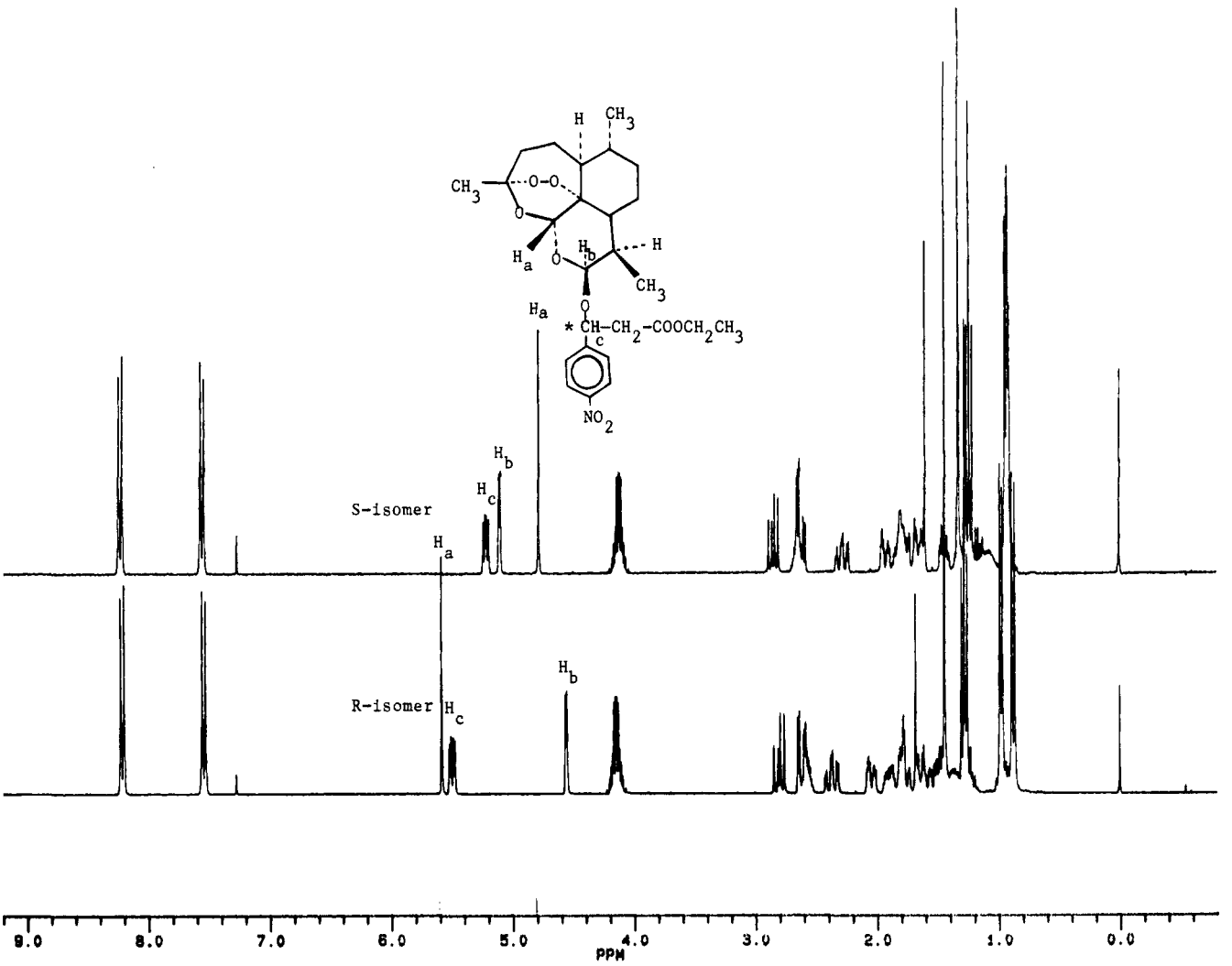


Figure 1. ^{1}H-NMR of **8h** (*R*) and **8i** (*S*).

compounds of this study the benzene ring has higher priority than the alkyl or carboxyalkyl functions. Therefore, the conformation of **8c** (*R*-isomer) is similar to **8j** (*S*-isomer), and **8d** (*S*-isomer) resembles **8h** (*R*-isomer) in NMR spectra. The minimum differences in chemical shifts of C_{10}-H (Δ 0.03 ppm) between **8a** (*R*) and **8b** (*S*) and the large differences (1 ppm) in C_{12}-H resonances suggested that the conformation of compounds **8a,b** which possess a benzylic group instead of a benzene ring is somewhat different from the rest of the compounds. The benzene ring in both *R*- and *S*-isomers (**8a,b**) appears to shield C_{10}-H to the same degree but to a lesser extent than to that of the other compounds. On the other hand, the benzene ring shielded the C_{12}-H in **8b** to a larger degree than the rest of compounds of this study. It is interesting to note that the benzene rings of this series also exert a shielding effect to the C_9-CH_3 protons of *R*-isomers, causing an increase in chemical shift differences between *R*- and *S*-isomers from less than 0.05 to 0.15 ppm. An understanding of the relationship between the conformation and NMR spectra based on the study of **8h,i** has made the structure assignment of all other single diastereomers of this study rather straightforward, based on the NMR of the single diastereomer alone.

Results and Discussion

The new dihydroartemisinin derivatives were tested *in vitro* against two clones of human malaria, *P. falciparum* D-6 (Sierra Leone I clone) and W-2 (Indochina clone). The IC_{50}'s shown in Table 3 are the average value of at least three experiments. All compounds were tested side by side in the same experiments. The former clone is a strain that is resistant to mefloquine and the latter to chloroquine, pyrimethamine, sulfadoxine, and quinine. These new compounds are

Table 3. *In Vitro* Antimalarial Activity Against *Plasmodium falciparum*

compd	IC_{50} (ng/mL) [artemisinin index]	
	W-2	D-6
artemisinin	1.0150 [1.00]	2.3020 [1.00]
artelinic acid	2.0818 [0.49]	4.8659 [0.49]
artemether	0.3008 [3.36]	0.8689 [2.65]
8a (*R*)	1.9158 [0.53]	3.0490 [0.75]
8b (*S*)	0.6968 [1.45]	0.9722 [2.37]
8c (*R*)	0.0938 [10.82]	0.2872 [8.01]
8d (*S*)	0.2265 [4.48]	0.5093 [4.52]
8e (*R*)	1.1475 [1.13]	1.4580 [0.63]
8f (*R*)	0.2134 [4.73]	0.4957 [4.64]
8g (*S*)	0.1437 [7.03]	0.4593 [5.01]
8h (*R*)	0.2297 [4.40]	0.5629 [4.09]
8i (*S*)	0.0487 [20.74]	0.2463 [9.34]
8j (*R*)	0.3368 [3.00]	0.9210 [2.50]
8k (*S*)	0.3353 [3.01]	0.6921 [3.30]
9a (*R*)	2.5350 [0.40]	5.7720 [0.40]
9b (*S*)	1.2308 [0.82]	2.9360 [0.78]
9c (*R*)	1.3470 [0.75]	2.8570 [0.81]

α-substituted benzylic ethers of dihydroartemisinin, with the exception of **8a,b** which are phenethyl ether instead of benzylic ether. All compounds possess either alkyl (CH_3 or $CH_2CH_2CH_3$), carbethoxyalkyl, or its corresponding acid ($CH_2COOCH_2CH_3$, CH_2COOH, or $COOCH_2CH_3$) substituents at the α-methylene carbon of the benzylic side chain. The substituents on the benzene ring are electron-withdrawing (COOH, $COOCH_3$, CF_3, or NO_2) functions.

All compounds listed in this study showed activity at least equal to or better than that of artelinic acid against *P. falciparum*. As was observed with the existing derivatives of this class, these new compounds showed higher inhibitory activity against W-2 than D-6 clones. Compound **8i** (IC_{50} = 0.0487 ng/mL against W-2 and 0.2463 against D-6), which is the most active of this class, showed inhibitory activity about 10-, 20-, and 40-fold better than artemether, artemisinin, and artelinic acid, respectively. The fact that compounds **8e,j,k** which have a small methyl group substituted at the α-methylene carbon showed weaker activity than the compounds with a carbethoxyalkyl substituent such as **8c,d,f–i** indicated that the lipophilicity and the steric effects of the molecule play an important role in their antimalarial activity. This fact is further substantiated by the significantly weaker antimalarial activity of the carboxylic acids **9a–c** than their corresponding esters **8j,k,h**, respectively. Furthermore, since compound **8i** (with a NO_2 substituent) is 3-fold more potent than the unsubstituted **8g**, the electronic effect also contributed significantly to the activity. The substantial differences in conformation between the *R*- and *S*-diastereomers, as indicated by the NMR and X-ray data, also reflected differences in their antimalarial activities. The *S*-isomers in general are severalfold more potent than the corresponding *R*-isomers. The differences in antimalarial activity observed with the *R*- and *S*-diastereomers suggest the possibility of the involvement of enzymatic bioactivation as a mode of action.

The design of this class of compounds is based on the chemical modifications of artelinic acid and its precursor, methyl artelinate which is a benzylic ether of dihydroartemisinin. Both methyl artelinate and artelinic acid showed moderate *in vitro* and *in vivo* antimalarial activities. This study showed that introduction of alkyl, carbethoxy, or carbethoxyalkyl function at the α-methylene position greatly improved the antimalarial activity. Likewise, introduction of an electron-withdrawing NO_2 group in the benzene ring enhances the antimalarial potency. The enhancement in antimalarial activity may be a result of the increase in lipophilicity of the molecule and the steric effect of the α-substituents on the methylene function. On the basis of the antimalarial data of the existing artemisinin derivatives, the *in vitro* antimalarial activity, in general, parallels the *in vivo* activity. The conversion of an ester derivative, such as **8h**, to its corresponding acid concomitantly decreased its antimalarial activity. However, the high potency of the new esters may give corresponding acids with higher efficacy than artelinic acid. In fact, compounds **9b,c** are twice as active as artelinic acid.

The structure–activity relationship of this study clearly indicated that, besides the peroxide function of the dihydroartemisinin moiety which was reported to be essential for antimalarial activity of this class of compounds, the lipophilicity of the molecule, the steric and electronic effect of the substituents, and the conformation of the side chain all play important roles in the antimalarial efficacy.

Experimental Section

Chemistry. All melting points were determined on a Mettler FP-62 melting point apparatus. Infrared spectra were obtained on a Nicolet 20SXB FT-IR spectrometer. NMR spectra were determined on a Brucker AC300 spectrometer with Me_4Si as an internal standard. Specific optical rotation was assessed on a Jasco DIP-360 digital polarimeter. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA, and the results are within 0.4% of the theoretical values, except where noted.

Condensation of Dihydroartemisinin (3) with Alcohols. Dihydroartemisinin[1] (**3**; 0.5 g, 1.8 mmol) was dissolved in 70 mL of anhydrous Et_2O. To the solution was added 3 mmol (excess) of an appropriate alcohol followed by 0.25 mL of $BF_3{\cdot}Et_2O$. The reaction mixture was stirred at room temperature for 24 h, washed successively with 5% aqueous $NaHCO_3$ and H_2O, dried over Na_2SO_4, and evaporated to dryness under reduced pressure. The resultant crude products were purified with a silica gel chromatotron, preparative TLC, or silica gel column using EtOAc/hexane or petroleum ether as eluant to give the pure single *R*- and *S*-diastereomers. The physical properties of these compounds and their ^{1}H-NMR data are listed in Tables 1 and 2, respectively.

Conversion of Esters 8 to the Corresponding Carboxylic Acids 9. Ester **8** (1 g) was dissolved in 30 mL of 2.5% KOH/MeOH and allowed to stand at room temperature for 2 days. To the solution was added an equal volume of H_2O (30 mL), and the MeOH was removed under reduced pressure. Upon cooling in an ice bath, the aqueous solution was acidified with dilute HCl and extracted three times with Et_2O. The ether extracts were combined, dried over Na_2SO_4, and evaporated to dryness. The residue was recrystallized from appropriate solvents (Table 1). The NMR spectra are shown in Table 2.

Biology. *In Vitro* Antimalarial Studies. The *in vitro* assays were conducted using a modification of the semiautomated microdilution technique of Desjardins et al.[22] and Chulay et al.[23] Two *P. falciparum* malaria parasite clones, from CDC Indochina III (W-2) and CDC Sierra Leone I (D-6), were utilized in susceptibility testing. They were derived by direct visualization and micromanipulation from patient isolates obtained from the Centers for Disease Control, Atlanta, GA, and cloned by Oduola et al.[24] in 1980 and 1982, respectively. The patients had acquired infections either in Vietnam or Sierra Leone. The W-2 clone is susceptible to mefloquine but resistant to chloroquine, sulfadoxine, pyrimethamine, and quinine, whereas the D-6 clone is naturally resistant to mefloquine but susceptible to chloroquine, sulfadoxine, pyrimethamine, and quinine. Test compounds were initially dissolved in DMSO and diluted 400-fold in RPMI 1640 culture medium supplemented with 25 mM Hepes, 32 mM $NaHCO_3$, and 10% human plasma. These solutions were subsequently serially diluted 2-fold with a Biomeck 1000 instrument (Beckman, Fullerton, CA) over 11 different concentrations. The parasites were preexposed to the compounds for 48 h and incubated at 37 °C with 5% O_2, 5% CO_2, and 90% N_2 prior to the addition of [^{3}H]hypoxanthine. After a further incubation of 18 h, parasite DNA was harvested from each microtiter well using a MACH II cell havester (Tomtec, Orange, CT) onto glass filters. Uptake of [^{3}H]hypoxanthine was measured with a 1205 Betaplate liquid scintillation counter (Wallac, Turku, Finland). Concentration–response data were analyzed by a logistic dose–response model, and the IC_{50} values (50% inhibitory concentrations) for each compound were calculated. Each measurement was performed at least three times, and the arithmetic means are shown in Table 3.

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Supplementary Material Available: Table of the chemical shifts of compounds **8** and **9** (2 pages). Ordering information is given on any current masthead page.

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