A series of artesinin (1) derivatives containing bromo and heterocyclic or aromatic amine functions was prepared in the search for analogues with good water solubility and high antimalarial activity. Treatment of dihydroartesinin (2a) with boron trifluoride etherate at room temperature gave the key intermediate, 9,10-dihydroartesinin (3), which, on reaction with bromine, gave the dibromide 4. The latter was condensed with amines in anhydrous CHCl₃ at 0°C to give the desired products in 25-55% yield. The new derivatives, tested in vitro against Plasmodium falciparum, were found to be more effective against W2 than D-6 clones and were not cross-resistant with existing antimalarials. Compound 6b, 3-fluoroaniline derivative, was the most active of the series, with the IC₅₀ < 0.16 µg/mL, making it several fold more potent than 1. However, no significant in vivo antimalarial activity against Plasmodium berghei was observed in any of the new compounds tested.
Antimalarial Activity of New Water-Soluble Dihydroartemisinin Derivatives. 3.1
Aromatic Amine Analogues

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A series of artemisinin (1) derivatives containing bromo and heterocyclic or aromatic amine functions was prepared in the search for analogues with good water solubility and high antimalarial activity. Treatment of dihydroartemisinin (2a) with boron trifluoride etherate at room temperature gave the key intermediate, 9,10-dehydrodihydroartemisinin (3), which, on reaction with bromine, gave the dibromide 4. The latter was condensed with amines in anhydrous CH2Cl2 at ≤ 10°C to give the desired products in 25-55% yield. The new 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. Compound 6b, 3-fluorooxazine derivative, was the most active of the series, with the K50 ≤ 0.16 ng ml, making it several fold more potent than 1. However, no significant in vivo antimalarial activity against Plasmodium berghei was observed in any of the new compounds tested.

Artemisinin (qinghaosu, arteannuin, 1), an antimalarial agent isolated from the plant Artemisia annua, is an endoperoxide-containing sesquiterpene lactone.1,2 The unusual chemical structure of artemisinin coupled with its low toxicity and proven antimalarial efficacy have attracted attention from both chemists and parasitologists since its discovery in 1972. The practical use of artemisinin as an antimalarial agent, however, is impaired by (a) its insolvibility in both water and oil,8 (b) its poor efficacy by oral administration,9 and (c) the rate of recrudescence in treated patients.9

The lactol form of 1, dihydroartemisinin (2a), prepared by the sodium borohydride reduction of the parent com-

ound, was shown to be more active than 1.6,7 But, because of its heminetal structure, the compound is believed to have lower stability than 1.

Chemical modifications of dihydroartemisinin to improve its efficacy and solubility have been reported by several laboratories. Artemether8-11 and arteether,12-14 the methyl and ethyl ethers of 2a, respectively, are more lipophilic and more effective than artemisin. Sodium artemate15-18, a water-soluble derivative of 2a, was demonstrated to be particularly useful in the treatment of cerebral malaria.19 A half-ester of succinic acid, sodium artemate is, however, not stable in aqueous solution, a property that is detrimental to its practical utility as an antimalarial agent.20

Recently, we prepared a series of derivatives of dihydroartemisinin (2a) in which the solubilizing group, carboxylate, was coupled to dihydroartemisinin by an alkyl ether rather than an ester linkage.19-22 Among the water-soluble derivatives that we prepared, the sodium salt of 2a was found to be equally active as the parent artemisinin and sodium artemate (2b) in vitro and more active than 1 and 2b in rodent Plasmodium berghei test systems. Furthermore, sodium artemate is substantially more stable than sodium artemate in weakly alkaline aqueous solution, an important physical property for the preparation of an intravenous injection dosage form. Antimalarial studies have shown that sodium artemate totally eliminated the parasitemia in mice infected with P. berghei when administered in their drinking water.23

In our continuing search for new artemisin analogues with good water solubility and high antimalarial efficacy, we report here the preparation and antimalarial studies of additional dihydroartemisinin derivatives which contain bromo and heterocyclic or aromatic amine functions. Water solubility, it was anticipated, would be achieved through salt formation.

**Chemistry**

The starting material, dihydroartemisinin (2a), was prepared by sodium borohydride reduction of 1 according to a modified literature procedure.22 Experiments have shown that proper adjustment of the pH of the reaction mixture before workup is critical to obtaining acceptable yields of product.

Dihydroartemisinin, upon treatment with boron trifluoride etherate at room temperature, gave a key intermediate, 9,10-dehydrodihydroartemisinin (3) in 75-80% yield (Scheme 1). Compound 3 was reported earlier to be a minor product when dihydroartemisinin was treated with alcohols under the boron trifluoride etherate catalysis and is probably formed by tautomerization of the oxonium intermediate, as discussed in the previous report.22
Treatment of 3 with bromine at low temperature gave a good yield of the corresponding dibromide (4). Inasmuch as two asymmetric carbons are created during the bromination, four possible dibromide isomers are theoretically possible. However, 4 was found to be unstable at room temperature and, therefore, was used for reactions with amines without purification.

Condensation of dibromide 4 with amines was carried out in anhydrous dichloromethane at \(-10^\circ\text{C}\). Because this reaction involves the formation of 1 equiv of HBr, 2 equiv of amine were necessary for each equivalent of dibromide employed. Purification of the crude product was achieved through silica gel chromatography and yields ranged from 25-55% (Table I).

In contrast to aromatic amines, aliphatic amines such as benzylamine, butylamine, and N,N-diethylhexylenediamine, gave no desired product under identical conditions. Instead, the major isolable product was 9,10-dehydrodihydroartemisinin (5) which was also found to be the minor product of reactions between aromatic amines and 4.

Since two asymmetric carbon centers \(C_8\) and \(C_9\) are created during the process of conversion from 3 to 6 and 7, four stereoisomers are possible in the final products of each reaction. However, only two isomers were isolated in reactions with 3-fluorotoluene and 2-aminothiazole were to give the observed products 6a-e and 7a-b.

Steric hindrance of the \(\alpha\) side of the intermediate 5 caused the preferential attack by the amine from \(\beta\) side which accounts for the observation that compound 6 is the major or the sole product of the condensation reactions. Like the ether formation of dihydroartemisinin with an alcohol under the catalysis of boron trifluoride etherate which gave mainly the \(\beta\) isomer and was also involved the oxonium ion as the intermediate, \(2\) the \(C_{10}\)N configuration is also \(\beta\) in both products 6 and 7.

A molecular model indicated that, due to steric hindrance by the surrounding functional groups, the rotation of the bulky heterocyclic or aromatic ring of the new compounds along the \(C_8\) and \(N\) bond is restricted and, thus, only two conformations are possible for \(N\)-H, with the amine proton skewed between both protons at \(C_9\) (\(\alpha\)-H and \(\beta\)-H) or between the \(C_9\)-H and the \(\beta\)-C\(_{10}\)-H. The latter conformation gave larger \(J\) value than the former conformation due to larger dihedral angle between \(C_9\)-H and the \(N\)-H. The \(J\) value on \(C_{10}\)-H (Table II) suggested that compounds 6a-f assume the latter conformation whereas compounds 7a-b have the former conformation. The chemical shift and the coupling constant of \(C_{10}\)-H and \(C_{10}\)-NH of the final products were established by \(D_2O\) exchange technique.

The structure determination of 6d was confirmed by X-ray study. The results of the study are illustrated in Figure 1. The absolute configuration of 6d \((C_{10},\ C_8\) and \(C_9\) are \(S\) and \(C_8\), \(C_{12}\), \(C_{20}\), and \(C_{6a}\) are \(R\)) agrees with that found for artemisinin \((1)^{15}\) With the exception that the brominated six-membered ring is more boat-shaped than

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Table III. In Vitro Antimalarial Activity of Dihydroartemisinin Derivatives against Plasmodium falciparum

<table>
<thead>
<tr>
<th>Compd</th>
<th>Artemisinin D-6</th>
<th>Indochina clone W-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a</td>
<td>0.91</td>
<td>0.23</td>
</tr>
<tr>
<td>6b</td>
<td>&lt;0.16</td>
<td></td>
</tr>
<tr>
<td>6c</td>
<td>3.42</td>
<td>0.63</td>
</tr>
<tr>
<td>6d</td>
<td>7.72</td>
<td>1.19</td>
</tr>
<tr>
<td>6e</td>
<td>20.98</td>
<td>5.36</td>
</tr>
<tr>
<td>1</td>
<td>0.16</td>
<td>0.01</td>
</tr>
</tbody>
</table>

chair-shaped, the overall conformation of the fused ring system in 6d is similar to that found for both artemisin and its derivatives such as arteether and dihydroartemisinin (2a). The reaction mixture was allowed to stand at room temperature for 2 h. The reaction mixture was then applied to a silica gel column chromatography using hexane and EtOAc as eluent as eluent to give the desired final products. Table I.

Results and Discussion

The new derivatives were tested in vitro against clones of human malaria, P. falciparum D-6 (Sierra Leone clone) and W-2 (Indochina clone). The former is a clone that is resistant to mefloquine and the latter, to chloroquine, pyrimethamine, sulfadoxine, and quinine. 

The results (Table III) indicate that the new derivatives, like the parent agent 1, are not cross-resistant with any of the antimalarial agents mentioned. As was observed with other water-soluble dihydroartemisinin derivatives, these new agents are more effective against W-2 than D-6. Compound 6b, 3-fluoromethane derivative, was the most active compound of the series, with the IC₅₀ (50% inhibitory concentration) of 0.16 μg/mL, making it several fold more potent than artemisinin. Compounds 6a and 6c showed activity comparable to artemisin, whereas 6d and 6e are less active than the parent compound.

Despite the in vitro activities against P. falciparum observed with 6a, c, no significant antimalarial activity against resistant malaria P. berghei in vitro was observed in any of the new compounds tested. No antimalarial testing was conducted on 7a due to insufficient supply of these isomers.

Experimental Section

Chemistry. All melting points were determined on a Thomas-Heath melting point apparatus and are uncorrected. Infrared spectra of solid samples were obtained in KBr disks on a Nicolet 20SX FT-IR spectrometer. NMR spectra were determined on a Varian spectrometer with the Cetus Pro! Pette (Perkin-Elmer Corp., Norwalk, CT). The structure was solved by direct methods as implemented by the SHELXL program. Full-matrix least-squares refinement on 259 parameters converged to a goodness of fit parameter of 1.4, and the final difference map was featureless.

Biology. (a) In Vitro Antimalarial Studies. The in vitro assays were conducted with use of the semiautomated microdilution technique of Desjardins et al. as modified by Milhous et al. Two P. falciparum malaria clones (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 Sierra Leone. The Indochina clone is resistant to the antimalarials chloroquine, sulfadoxine, pyrimethamine, and quinine, whereas the Sierra Leone, 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 × 10⁻⁴ μmol. Parasite inocula at 0.5% parasitemia and 1% hematocrit were incubated for 24 h and added to equilibrium concentrations of each test compound prior to the addition of H⁺-pyrazolone. After a further incubation

(1) Sheldrick, G. M. SHELXL, Minicomputer Programs for Structure Determination; University of Göttingen: Göttingen, West Germany, 1980.
of 18 h, particulate matter was harvested from each microtiter well with use of 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 curative activities of these new compounds were measured in a test where mice were infected with \(5.98 \times 10^5\) \(P.\) berghei parasitized cells intraperitoneally on day 0. Test compounds were dissolved in peanut oil and were administered subcutaneously once a day for three consecutive days commencing on day 3. The dose levels of compounds given were 640, 160, 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.

Acknowledgment. We thank Dr. Arba L. Ager, Jr., University of Miami, for performing the in vivo, and Dr. Wilbur Milhous of WRAIR for the in vitro antimalarial studies. This work was supported, in part, by the Office of Naval Research.

Registry No. 1, 63968-64-9; 29, 71939-50-9; 3, 8296-30-3; 4, 127971-91-9; 6f, 127971-92-0; 6e, 127997-42-6; 6c, 127997-43-7; 6d, 127971-93-1; 6e, 127971-94-2; 6f, 127971-95-3; a, 128050-94-2; 7b, 128052-60-8; 3-FCO\(_2\)H\(_2\)NH\(_2\), 372-19-0; C\(_6\)H\(_2\)NH\(_2\), 62-53-3; 2-aminothiazole, 96-56-4; 2-aminopyridine, 504-29-0; 2-aminopyrimidine, 109-12-0.

Supplementary Material Available: Tables of atomic coordinates, bond lengths, bond angles, anisotropic displacement coefficients, and H-atom coordinates for compound 6d (5 pages). Ordering information is given on any current masthead page. Tables of atomic coordinates and bond lengths and angles have been deposited with the Crystallographic Data Centre, Cambridge University Chemical Laboratory, Cambridge CB2 1Ew. England.