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Registry No. 2, 64757-13-7; 3, 112114-02-0; 4, 112114-03-1; 4·oxalate, 112114-12-2; 5, 10447-21-9; 7, 112114-04-2; 7·HCl, 112114-13-3; 8, 112114-05-3; 8·maleate, 112114-14-4; 9, 112114-06-4; 10, 112114-07-5; 10·oxalate, 112114-15-5; 11, 59311-67-0; 12, 28783-50-8; 13, 104535-60-6; 14, 112114-08-6; 14·HCl, 112114-16-6; 15, 97456-68-3; 16, 97456-71-8; 17, 112114-09-7; 17·maleate, 112114-17-7; 18, 29970-79-4; 18·HCl, 112114-18-8; 19, 4802-79-3; H_2C=CHC(OTMS)=CH_2, 38053-91-7; 2-(2-bromoethyl)-1,3-dioxolane, 18742-02-4; *cis*-ethyl 2-(2-(1,3-dioxolan-2-yl)ethyl)-octahydro-1-oxoquinolizine-2-carboxylate, 112114-10-0; *trans*-ethyl 2-(2-(1,3-dioxolan-2-yl)ethyl)octahydro-1-oxoquinolizine-2-carboxylate, 112114-11-1; 2-hydrazinopyridine, 4930-98-7; 3-(2-formamidoethyl)thiophene, 28783-48-4.

Arteether, a New Antimalarial Drug: Synthesis and Antimalarial Properties

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Arteether (6) has been prepared from dihydroqinghaosu (3) by etherification with ethanol in the presence of Lewis acid and separated from its chromatographically slower moving α-dihydroqinghaosu ethyl ether (7). The absolute stereochemistry at C-12 has been determined by ^{1}H NMR data ($J_{11,12}$, NOESY). Ethyl ethers 6 and 7 showed potent in vitro inhibition of *Plasmodium falciparum*, and both compounds were highly potent antimalarials in mice infected with a drug-sensitive strain of *Plasmodium berghei*. Crystalline arteether (6) and its oily epimer 7 were 2–3 times more potent schizontocides than quinghaosu (1), but deoxy compounds 8, 9, and 11 were 100–300 times less potent in vitro than their corresponding peroxy precursors. Pharmacological studies have shown arteether (6) to have antimalarial activity in animals comparable to artesunate (2) and artemether (4), both of which are fast-acting blood schizontocides in humans. Arteether (6) has now been chosen for a clinical evaluation in high-risk malaria patients.

Qinghaosu (QHS, 1),[7] a sesquiterpene peroxide, is an active ingredient of *Artemisia annua* L. that has been used as an antimalarial preparation in China in the form of extracts for centuries.[8,9] Details on the isolation of QHS,[10] its structure determination,[11,12] and its antimalarial effects in infected animals[9,13] and malaria patients[9,13] have been reviewed.[13,14] QHS is only sparingly soluble in water or oils and not well absorbed by the gastrointestinal tract. A search for more potent analogues of QHS with better bioavailability was initiated in China, focusing attention on ethers and esters of dihydroquinghaosu (DQHS, 3).[15,16] DQHS, obtained by reduction of QHS with sodium borohydride, is an acetal and behaves in solution as a mixture of anomers.[17] Attention to possible clinical use of derivatives of QHS focused early on artesunate (2),[18] a sodium salt of a hemisuccinate of α-DQHS, which is soluble in water and was found to be a highly effective antimalarial in animal models.[19] Artesunate proved to be extremely sensitive to hydrolysis,[20] and therefore, it is unclear whether its pharmacological effects are due to the parent drug or its hydrolysis product DQHS. Artemether (4), on the other hand, an ether representative of the β-series,[17] obtained by etherification of DQHS with methanol in the presence of Lewis acids, was found to be much more stable, and when given by im injection in an oily solution to malaria patients, it had activity comparable to that of artesunate.[21]

The Steering Committee of the Scientific Working Group on Malaria Chemotherapy of the World Health Organization in Geneva, Switzerland (SWG-CHEMAL), responsible for the development of new antimalarial drugs,

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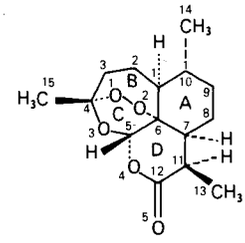
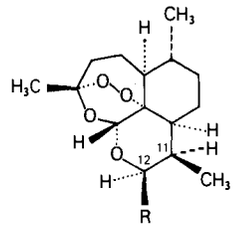
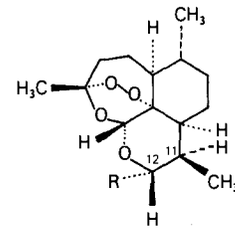


1 qinghaosu



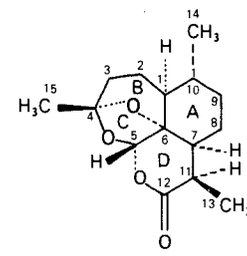
β-series



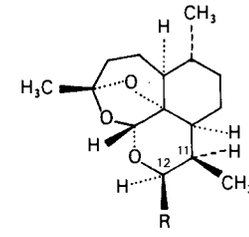
α-series

	R = O–CO–CH_2–CH_2–COOH	2
3	R = OH	
4	R = OCH_3	5
6	R = OCH_2CH_3	7

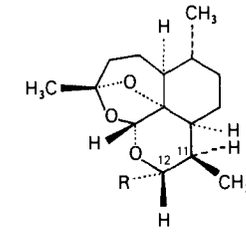
Figure 1.



8 Deoxyqinghaosu



β-series



α-series

9	R = OH	9
	R = OCODADF	10
11	R = OCH_2CH_3	12
13	R = OCH_3	

Figure 2.

decided early in 1985 to pursue actively the lead provided by QHS and to develop the β-ethyl ether isomer of DQHS (**6**) now called arteether,[22] for use in high-risk malaria patients including those with cerebral malaria.

This decision was based on the assumption that arteether (**6**) with an ethyl ether group instead of the similarly oriented methyl ether group in artemether (**4**) would be more lipophilic, a possible advantage for its accumulation in brain tissue. Arteether also was expected to afford ethanol by cleavage of the ethyl ether function and not methanol as does artemether, a fact not to be ignored with the relatively large dose of drug to be given (3 × 300 mg/day per patient). The finding that arteether was highly crystalline, whereas the α-DQHS ethyl ether **7** was not, provided an additional advantage in the large-scale preparation of chemically pure arteether over that of artemether, where both ether isomers are low-melting solids.

Chemistry

A. Arteether (6). Reduction of **1** (Figure 1) with an excess of sodium borohydride in methanol at 0–5 °C afforded **3** in 79% yield. When the reaction was carried out at room temperature, only 23% of **3** was obtained. Etherification of **3** in ethanol in the presence of a catalytic amount of boron trifluoride etherate at 70 °C during 1 h afforded a 3:1 mixture of arteether **6** and its α-DQHS ethyl ether **7** in nearly quantitative yield. Most of **6** could be separated from the oily derivative **7** by crystallization from hexane. The remaining α/β mixture was separated chromatographically on silica gel to give **7** in pure form. The conformations assigned to **6** and **7** were made on NMR spectral ground.[17,22] The configurations at C-12 of these compounds are assigned based on the vicinal couplings, $^3J_{11,12}$, which are generally dependent upon the orientations of coupled nuclei. The large $^3J_{11,12}$ (9.2 Hz) observed in **7** is indicative of a trans diaxial relationship between the H-11 and H-12, hence assigned to an α-configuration. The $^3J_{11,12}$ value of 3.6 Hz in **6**, on the other hand, suggested a gauche relationship between these two coupled protons, thus affording a β-configuration. The assigned structures are consistent with results of 2D NOESY experiments, which show a cross-peak (~5% NOE) between H-5 and H-12 for **7** but no cross-peak between these two protons for **6** in 2D contour plots. The isomerization of **7** into **6** was best achieved by heating the former for 3 h under the conditions used for the etherification of **3**. A mixture of **6** and **7** was obtained along with minor byproducts. Replacing the acid catalyst boron trifluoride etherate with *p*-toluenesulfonic acid or hydrochloric acid also resulted in the formation of **6** although in considerably lower yields.

B. Potential Metabolites of Arteether. Investigations by the Chinese with artemether (**4**) suggest two metabolic pathways: a major one affording deoxyartemether **13** by extrusion of an oxygen atom from the peroxide bridge,[23] and a minor one affording DQHS (**3**), shown as the β-isomer. With this information, and assuming that arteether (**6**) could have a similar metabolic behavior, several deoxy compounds were prepared to be used as internal standards to monitor **6** and its metabolic conversion (Figure 2). Catalytic reduction of QHS (**1**) over $Pd/CaCO_3$ in methanol and treatment of the reaction product with *p*-toluenesulfonic acid in benzene prior to crystallization gave deoxyQHS (**8**) already reported by Chinese investigators[11] and identical with material recently obtained by synthesis.[24] The lactone group in compound **8**, in contrast to **1**, was not affected by sodium borohydride in methanol. DeoxyDQHS (**9**) was, however, obtained by similar catalytic deoxygenation of DQHS **3** and found to be identical with material prepared elsewhere.[11] Ester **10**, detectable by UV spectroscopy (241 nm, log ε = 4.2) in nanomolar concentration, was prepared from **9** and diacetyldihydrofluorescein as reported.[25] Deoxyarteether

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Table I. Selected ^{1}H NMR Data (300 MHz, in $CDCl_3$) of Artesunate (**2**) to α-DeoxyDQHS Ethyl Ether (**12**)

	H–C(5), ppm		H–C(12), ppm		H–C(11), ppm		$^3J_{11,12}$, Hz		$^3J_{7,11}$, Hz	
compd	α	β	α	β	α	β	α	β	α	β
2[a]	5.40		5.77		2.56		9.8		4.3	
3[a,b]	(5.34)	5.56	(4.71)	5.26	(2.31)	2.56	(9.2)	3.1		
4[a]		5.35		4.65		2.57		3.4		
5[a]	5.32		4.32		2.36		9.3			
6		5.41		4.80		2.64		3.6		4.9
7	5.33		4.43		2.44		9.2		4.3	
9[b]	(5.35)	5.36	(4.77)	5.31	(2.32)	2.46	(6.3)	5.3	(4.8)	6.3
10	5.42		6.07		2.79		6.3			
11		5.31		4.79		2.42		4.5		5.4
12	5.29		4.43		2.40		6.1		5.0	

[a] These data are taken from ref 17. [b] The numbers in the parentheses are derived from spectra containing both the α- and β-species after equilibration.

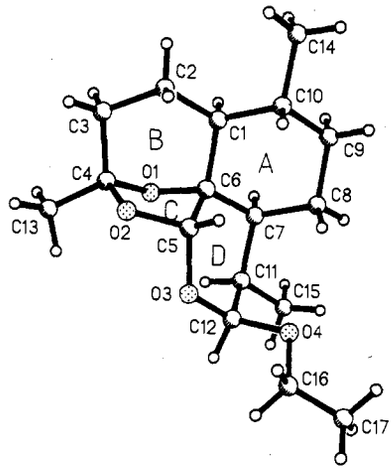

Figure 3. Diagram showing the structure and conformation of **11**. The figure is drawn by using the experimentally determined coordinates with arbitrary thermal parameters.

(**11**) and α-deoxyDQHS ethyl ether (**12**) were obtained from **6** and **7** by catalytic deoxygenation. ^{1}H NMR spectral analysis of these compounds indicated notable changes of the $^3J_{11,12}$ and $^3J_{7,11}$ coupling in going from the peroxide to the deoxy series (see Table I). Compounds **9**α and **12**, which show $^3J_{11,12}$ and $^3J_{7,11}$ of 6.1 and 5.0 Hz, respectively, were assigned to the α-isomers, whereas **9**β and **11** with $^3J_{11,12}$ and $^3J_{7,11}$ of 4.5–5.4 and 5.3–6.3 Hz, respectively, were assigned to β-isomers. The β-configuration assigned to **11** was further confirmed by the X-ray study.

C. X-ray Results Discussion. The results of the X-ray study on **11** are illustrated in Figure 3. Of the six-membered rings, A has a normal chair conformation while rings B and D have slightly distorted chair conformations (average absolute values for ring torsion angles range from 53.5 to 56.9° for ring A, 40.4 to 77.6° for ring B, and 39.0 to 54.5° for ring D). The five-membered ring has a twisted conformation such that O1 and C6 lie on opposite sides of the plane formed by C4, O2, and C5. All ring bond lengths fall into normal ranges; however, there is some strain in the molecule at the fusion point of rings B, C, and D as shown by the C1–C6–C5 angle of only 98.9 (3)°. The X-ray results confirmed the prediction that the CH_3 and OCH_2CH_3 moieties on ring D are cis to one another (C15–C11–C12–O4 = 53.2°).

Biological Evaluation

In Vitro Assessment of Antimalarial Activity. Both arteether (**6**) and its α-isomer **7** are potent antimalarials in two control *Plasmodium falciparum* clones (Table II), equipotent with artemether (**4**), and about 2 times more potent than the natural drug QHS (**1**). The Indochina clone is resistant to the antimalarial drugs chloroquine, pyrimethamine, sulfadoxine, and quinine but susceptible to mefloquine. The D-6 Sierra Leone clone is susceptible to chloroquine, pyrimethamine, and sulfadoxine but resistant to mefloquine.

Table II. 50% Inhibitory Concentrations (10^{-9} M)

	W-2 Indochina clone:		D-6 Sierra Leone clone:	
antimalarial drug		QHS[a] indices		QHS indices
arteether (**6**)	2.94	(2.18)	4.07	(2.58)
α-DQHS ethyl ether (**7**)	3.07	(2.08)	4.18	(2.52)
artemether (**4**)	3.34	(1.92)	4.49	(2.34)
QHS (**1**)	6.41	(1.00)	10.53	(1.00)
DQHS (**3**)	1.79	(3.58)	1.83	(5.75)
sodium artesunate (2-Na)	1.66	(3.86)	2.18	(4.83)
chloroquine	99.97	(0.06)	10.84	(0.97)
mefloquine	5.75	(1.11)	33.38	(0.31)

[a] QHS indices represent intrinsic equimolar activity of each drug relative to the simultaneous QHS control.

Table III. In Vitro Evaluation of Putative Deoxymetabolites of QHS and Analogues against the D-6 Sierra Leone Clone

compd	IC_{50}, ng/mL	MW	IC_{50}, 10^{-9} M	fold difference
QHS (**1**)	2.97	282	10.53	
				272
deoxyQHS (**8**)	761.58	266	2863.08	
DQHS (**3**)	0.52	284	1.83	
				277
deoxyDQHS (**9**)	135.62	268	506.04	
arteether (**6**)	1.27	312	4.07	
				142
deoxyarteether (**11**)	171.57	296	579.69	

Deoxy compounds **8**, **9**, and **11** similarly tested in vitro as blood schizontocides did not show noteworthy activity (Table III). This clearly supports the view expressed earlier[23] that the peroxy function is vital for expressing antimalarial activity and that release of "active" oxygen is immediately connected with the mode of action of QHS-related drugs.

Another potential metabolite is DQHS (**3**), measured in blood by HPLC using reductive electrochemical detection as the diacetyldihydrofluoresceyl derivative and shown by Luo et al. to be an extremely sensitive analytical probe.[25]

Blood Schizontocidal Activity in Mice. The results summarized in Table IV indicate that, against the drug-sensitive N strain parasites, α-DQHS ethyl ether and arteether had a similar level of activity, which was 2–4 times greater than that of QHS. Against the mildly chloroquine

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Table IV. Blood Schizontocidal Activity of α-DQHS Ethyl Ether, Arteether, and QHS in Vivo[a]

parasite	primary resistance to	α-DQHS ethyl ether		arteether		QHS	
		ED_{90}	I_{90}	ED_{90}	I_{90}	ED_{90}	I_{90}[b]
P. berghei N		1.3	1.0	0.5	1.0	2.3	1.0
P. berghei NS	chloroquine	1.5	1.3	2.2	2.0	10.0	2.4
P. berghei RC	chloroquine	16.5	13.8	8.2	7.5	630	150
P. berghei N/1100	mefloquine	4.1	3.4	4.2	3.8	17.0	4.0
P. berghei N/HAL	halofantrine	9.5	7.3	2.1	4.2	31.0	13.5
P. berghei QS	quinine	13.8	10.6	1.4	2.8	24.0	10.4
P. berghei NPN	pyronaridine	3.5	2.9	2.5	2.3	90.0	21.4
P. berghei P	primaquine	2.5	2.1	0.8	0.7	12.0	2.9
P. berghei B	cycloguanil	2.9	2.4	0.9	0.8	8.2	2.0
P. berghei PYR	pyrimethamine	1.3	1.1	0.8	0.7	4.8	1.1
P. berghei ORA	sulfaphenazole	0.8	0.7	0.8	0.7	7.5	1.8
P. berghei MEN	menoctone	1.7	1.4	2.2	2.0	6.2	1.5
P. berghei ART	QHS	29.0	22.3	2.3	4.6	49.0	21.3

[a] Data from "4-day test".[27] With the exception of the NS line, all lines are resistant to the maximum doses of the respective compounds tolerated by the host. [b] Doses given subcutaneously on each of 4 consecutive days.

resistant NS line, arteether, α-DQHS ethyl ether, and QHS were slightly less active than against the N strain, but arteether and α-DQHS ethyl ether proved far more active against the highly chloroquine resistant RC line, the I_{90} levels being 13.8, 7.5, and 150 for α-DQHS ethyl ether, arteether, and QHS, respectively; α-DQHS ethyl ether and QHS showed a much reduced activity against the quinine-resistant QS and the halofantrine-resistant N/HAL lines. The mefloquine-resistant N/1100 line was less resistant to all three compounds with I_{90}'s between 3.4 and 4.0. All three compounds were significantly less active against the QHS-resistant ART line, but with an I_{90} of only 4.6 for arteether, whereas it was 22.3 for α-DQHS ethyl ethher and 21.3 for QHS itself. α-DQHS ethyl ether and arteether showed activity against lines resistant to primaquine, cycloguanil, pyrimethamine, sulfaphenazole, and menoctone similar to that against the N strain, but QHS had reduced activity against the pyronaridine-resistant NPN line with an I_{90} of 21.4. Against all the resistant lines, arteether and α-DQHS ethyl ether were significantly more active than QHS.

Causal Prophylactic Activity in Mice. To test for activity against the preerythrocytic schizonts, mice that had received an intravenous inoculum of sporozoites of *Plasmodium yoelii nigeriensis* were given a single, subcutaneous dose of test drug 3 h postinfection. Blood films were examined daily for up to 14 days.[26] Activity was assessed by comparing the time required for parasitaemia to reach a level of 2% in treated animals as compared with appropriate controls.[26] This procedure permits the evaluation of true drug action as compared with one that may be due to residual compound acting on the first generation of intraerythrocytic parasites. In this test, arteether showed no activity up to a dose level of 30 mg/kg and only residual activity at this level. QHS was inactive up to 300 mg/kg and also showed only residual activity at that level.

Conclusions

Arteether (**6**), an ethyl ether derivative of DQHS (**3**), is a crystalline, stable, and highly lipophilic compound, ideally suited for im injection in oily solution. QHS, which is administered orally, and artemether, which is given as an oily solution by im injection, both have low toxicity in experimental animals.[9] Arteether (**6**), which is chemically closely related to the two compounds, can be expected to behave similarly. Although resistance to QHS in *P. yoelii* in mice was recently produced,[23] it is expected that QHS and its more potent analogues, particularly arteether, will be highly effective antimalarial agents, and because of their novel structure, these compounds will be useful additions to the classical arsenal of antimalarial agents. Preclinical toxicity studies with arteether in sesame oil are being initiated.

Experimental Section[28]

Chemistry. Melting points were determined in open capillary tubes on a Büchi 510 (a) or on a Fisher-Johns apparatus and are uncorrected. The optical rotations were measured on a Perkin-Elmer 141 (a) or 241 MC (b) polarimeters. IR spectra were recorded on a Perkin-Elmer 257 grating infrared spectrophotometer (a) or on a Beckman IR 4230 instrument (b). ^1H NMR and NOESY spectra were taken on a Varian XL-300 spectrometer with $(CH_3)_4Si$ as the internal reference. Chemical ionization (CI) mass spectra as m/z values were carried out on a Finnigan 1015D spectrometer and electron impact (EI) mass spectra on a V. G. MICROMASS LTD 7070F spectrometer. Elemental analyses were performed by Microlit Laboratories, Inc. (a) or by Atlantic Microlab, Inc. (b). Merck silica gel $60F_{254}$ analytical TLC plates, Merck silica gel 60 (230–400 and 70–230 mesh), and Aldrich aluminum oxide, activated, neutral, Brockmann I (150 mesh) for column chromatography were used throughout this work. UV spectra were taken on a Hewlett-Packard 8450 A UV/vis spectrometer. In all the reactions carried out under a dry atmosphere of N_2, the glassware material was oven dried, assembled while hot, and allowed to cool while being flushed with dry N_2.

Dihydroqinghaosu (DQHS, 3). To a stirred solution of QHS (1; 240 g, 0.85 mol) in MeOH (12 L), contained in a 50-L double-walled reaction vessel (from QVF Glastechnik GmBH) and maintained at 0–5 °C by using an ethylene glycol–H_2O mixture thermostated at −3 °C, was added $NaBH_4$ (240 g, 6.34 mol), over a period of 1.75 h. After being stirred for an additional 1.25 h under the same conditions, the mixture was neutralized with acetic acid (375 mL) while the temperature was maintained in the 0–5 °C range, concentrated by distilling off 8.5 L of solvent [45 °C/(400 mm)], diluted with cold H_2O (7.5 L), and stirred for 15 min at room temperature. The white precipitate was collected and washed with H_2O–MeOH (2:1, 0–5 °C, 2 × 700 mL). The filtrate was stored for 14 h at 4 °C. The small amount of precipitate formed was collected and washed with H_2O–MeOH (2:1, 0–5 °C, 2 × 30 mL). The wet crops were pooled and dissolved in CH_2Cl_2 (7 L). After drying (120 g $MgSO_4$) and evaporation of the solvent, 191.4 g (79%) of **3** was obtained, mp 149–453 °C (a). The material was identical with that reported in the literature.[11]

Arteether (6). A solution of **3** (191 g, 0.67 mol) in EtOH (750 mL) and benzene (2.25 L) was heated to 45 °C. After rapid addition of BF_3·Et_2O (9 mL, 0.071 mol), the mixture was refluxed

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(28) Samples of compounds 8–12 and DADFDQHS will be available on request from the secretary of SWG-Chemal, World Health Organization, 1211 Geneva 27, Switzerland.

for 1 h under N_2, washed with saturated NaOAc solution (2.4 L) and H_2O (2.25 L), dried (75 g of $MgSO_4$), and concentrated to give 210.1 g of a semisolid mixture of **6** and **7** (3:1), which was dissolved in hexane (135 mL) at 45 °C and stored at −20 °C for 36 h. The white crystalline precipitate was collected, washed with hexane (2 × 70 mL, <0 °C), and dried to give **6** (136.9 g, 65%), mp 80–82 °C (a). From the concentrated filtrate, a further 14.9 g (7%) of material was obtained, mp 79–81 °C. Total yield of **6** was 151.8 g (72%). Recrystallization of this material together with 351.4 g of **6** of the same quality[29] from hexane (330 mL, <0 °C, 24 h) afforded after washing with hexane (250 mL, <0 °C) 456.9 g of **6**, mp 80–82 °C (a). Concentration of the filtrate afforded an additional 36.6 g of material, mp 80–82 °C (a). The two crops were combined and dried [30 °C/(0.2 mm)] to give 493.5 g of pure arteether (**6**): mp (a) 80–82 °C; $[\alpha]^{21}_D$ (a) +154.5° (*c* 1.0, $CHCl_3$); IR (a) (KBr) ν_{max} 2980, 2960, 2880, 2855, 1451, 1378, 1033, 985, 874 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (d, J = 7.5, 3 H, CH_3 at C-11), 0.95 (d, J = 6.2, 3 H, CH_3 at C-10), 1.20 (t, J = 7.5, 3 H, OCH_2CH_3), 1.45 (s, 3 H, CH_3 at C-4), 1.10–2.15 (m, 10 H), 2.38 (dt, 1 H), 2.64 (m, 1 H, H-11), 3.48 and 3.88 (m, OCH_2CH_3), 4.80 (d, J = 3.6, 1 H, H-12), 5.41 (s, 1 H, H-5); MS (a), m/z 313 (M^+ + 1). Anal. (a) ($C_{17}H_{28}O_5$) C, H.

α-Dihydroqinghaosu Ethyl Ether (α-DQHS Ethyl Ether, 7). Portionwise chromatography of the combined residues (185 g) recovered from all available mother liquors[29] on a 20-fold amount of silica gel in hexane–EtOAc (9:1) led to an additional 40 g of **6** and 126.9 g of **7** contaminated with a trace of **6**. A sample was rechromatographed to give pure **7**: $[\alpha]^{21}_D$ (a) −2.8° (*c* 1.0, $CHCl_3$); IR (a) ($CHCl_3$) ν_{max} 2930, 2880, 1380, 1050, 1016, 877 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (d, J = 7.5, 3 H, CH_3 at C-11), 0.95 (d, J = 6, 3 H, CH_3 at C-10), 1.21 (t, J = 7.5, 3 H, OCH_2CH_3), 1.44 (s, 3 H, CH_3 at C-4), 1.10–2.20 (m, 10 H), 2.39 (m, 1 H), 2.44 (m, 1 H, H-11), 3.51 and 4.01 (m, OCH_2CH_3), 4.43 (d, J = 9.2, 1 H, H-12), 5.33 (s, 1 H, H-5); MS (a), m/z 313 (M^+ + 1).

Epimerization of 7 into 6. To a solution of **7** (5.13 g, 16.4 mmol) in benzene (60 mL) and EtOH (20 mL) was rapidly added $BF_3{\cdot}Et_2O$ (240 μL, 1.9 mmol), and the resulting mixture was refluxed under N_2 for 3 h, washed with saturated NaOAc solution (65 mL) and H_2O (60 mL), dried ($MgSO_4$), and concentrated to give 5.01 g of **6** and **7** (2:1). Crystallization from hexane (3 mL) afforded **6** (1.66 g, 32%), mp (a) 79–80 °C. The filtrate was concentrated, and the residue was chromatographed on 200 g of silica gel in hexane–EtOAc (9:1) to yield a further 1.28 g (25%) of **6** and 1.48 g (29%) of **7**.

Deoxyqinghaosu (DeoxyQHS, 8).[11] To a solution of QHS (**1**; 434 mg, 1.54 mmol) in methanol (25 mL) was added 5% $Pd/CaCO_3$ (20 mg), and the mixture was hydrogenated at room temperature and atmospheric pressure. When the starting material disappeared by TLC (silica gel, ethyl acetate–hexane, 1:5), the reaction mixture was filtered through Celite. The residue obtained after evaporation was dissolved in benzene (10 mL), *p*-toluenesulfonic acid (10 mg) was added, and the solution was stirred overnight at room temperature. The benzene was evaporated, and the extract was taken up in ethyl ether, washed with distilled water, dried with Na_2SO_4, and concentrated under vacuum to give **8** as a white solid (383 mg, 94% yield). Crystallization from ethyl ether–hexane gave colorless prisms (350 mg, 86% yield): mp (b) 111–113 °C (lit.[11] mp 105–107 °C); $[\alpha]^{22}_D$ (b) −136° (*c* 0.97, $CHCl_3$) [lit.[11] $[\alpha]^{22}_D$ −133.7° (*c* 0.98, $CHCl_3$)]; MS (b), m/z 266 (M^+); ^1H NMR (b) ($CDCl_3$) δ 0.95 (d, J = 6.0, 3 H, CH_3 at C-10), 1.20 (d, J = 7.4, 3 H, CH_3 at C-13), 1.53 (s, 3 H, CH_3 at C-4), 3.19 (m, 1 H, H-11), 5,7 (s, 1 H, H-5); IR (b) (KBr) ν_{max} 2950, 1750 cm^{-1}.

Deoxydihydroqinghaosu (DeoxyDQHS, 9):[11] mp (b) (acetone–hexane) 142–144 °C (lit.[11] mp 142–143 °C); $[\alpha]^{22}_D$ (b) −43.1° (*c* 1.2, $CHCl_3$) [lit.[11] $[\alpha]^{22}_D$ −44.2° (*c* 0.95, $CHCl_3$)]; MS (b), m/z 268 (M^+); ^1H NMR (b) ($CDCl_3$) δ α-form 2.32 (m, 1 H, H-11), 4.77 (d, J = 6.3, 1 H, H-12), 5.35 (s, 1 H, H-5), β-form 2.46 (m, 1 H, H-11), 5.31 (d, J = 5.3, 1 H, H-12), 5.36 (s, 1 H, H-5); IR (b) ν_{max} 3420, 2950 cm^{-1}.

12α-(Diacetyldihydrofluoresceyl)deoxydihydroqinghaosu (α-DADFdeoxyDQHS, 10):[25] mp (b) 98–104 °C (lit.[25] mp 106–108 °C); $[\alpha]^{25}_D$ (b) −60.4° (*c* 0.38, $CHCl_3$), [lit.[25] $[\alpha]^{25}_D$ −68.19°); MS (b), m/z 668 (M^+); UV 241 nm (log ε = 4.2) ($CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.93 (d, 3 H, J = 5.1, CH_3 at C-10), 1.12 (d, 3 H, J = 7.1, CH_3 at C-11), 2.28 (s, 6 H, 2 AcO), 2.79 (m, 1 H, H-11), 5.42 (s, 1 H, H-5), 6.07 (d, 1 H, J = 6.3, H-12), 6.47 (s, 1 H, DADF-CH), 6.72–7.89 (m, 10 H, 10 ArH); IR (KBr) ν_{max} 2920, 1760, 1710, 1600, 1575, 1480, 1190, 1140, 1000 cm^{-1}.

Deoxyarteether (11). To a solution of **6** (770 mg, 2.47 mmol) in ethanol (20 mL) was added 5% $Pd/CaCO_3$ (20 mg), and the mixture was hydrogenated at room temperature and atmospheric pressure. The reaction mixture was filtered through Celite and concentrated. The residue obtained was extracted with ethyl ether (10 mL), washed with distilled water (5 mL), and dried over Na_2SO_4. Filtration through a column of alumina gave deoxyarteether (**11**) as a white solid (580 mg, 79%), crystallized from ethanol (69%): mp (b) 71–72 °C; $[\alpha]^{22}_D$ +7.3 (*c* 1.1, $CHCl_3$); MS (b), m/z 296 (M^+); ^1H NMR ($CDCl_3$) δ 0.89 (d, J = 5.8, 3 H, CH_3 at C_{10}), 0.91 (d, J = 7.6, 3 H, CH_3 at C-11), 1.18 (t, J = 7.1, 3 H, CH_2CH_3), 1.52 (s, 3 H, CH_3 at C-4), 2.42 (m, 1 H, H-11), 3.44 (m, 1 H, OCH_2CH_3), 3.83 (m, 1 H, OCH_2CH_3), 4,79 (d, J = 4.5, 1 H, H-12), 5.31 (s, 1 H, H-5); IR (KBr) ν_{max} 2950, 1450, 1380, 1020, 980 cm^{-1}. Anal. (b) ($C_{17}H_{28}O_4$) C, H.

X-ray crystallographic data for 11: $C_{17}H_{28}O_4$, molecular weight = 296.40, monoclinic, space group $C2$, a = 17.801 (5) Å, b = 10.582 (4) Å, c = 9.707 (2) Å, β = 112.79 (2)°, d_{calcd} = 1.17 g cm^{-3}, Z = 4, μ = 0.08 mm^{-1}, 1417 independent reflections were measured out to $2\theta_{max}$ = 48° with a Nicolet R3M diffractometer using Mo Kα radiation (λ = 0.710 69 Å) with a graphite monochromator in the incident beam. The data were collected at room temperature by using the $\theta/2\theta$ scan technique with a variable scan rate ranging from 15°/min minimum to 30°/min maximum, depending upon the intensity of a reflection. The structure was solved by direct methods as implemented by the SHELXTL system of programs.[30] Full-matrix least-squares refinement on 201 parameters (coordinates and anisotropic thermal parameters for non-hydrogen atoms; hydrogen atoms originally placed at calculated positions and then allowed to ride on covalently bonded atoms, that is, C–H distances set at 0.96 Å and coordinate shifts of attached C atom also applied to H atom, C–C–H angle set as close to 109.5° as possible, methyl groups were also allowed to torsion about the C–C single bonds), using the 1063 reflections for which $|F_o| > 3\sigma|F_o|$ gave a final R factor of 5.7% (R_w = 5.1%). The goodness-of-fit parameter was 1.67, and the final difference map was featureless. 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.

α-Deoxydihydroqinghaosu Ethyl Ether (α-DeoxyDQHS Ethyl Ether, 12). A solution of **7** (700 mg, 2.37 mmol) in ethanol (15 mL) was hydrogenated over 5% $Pd/CaCO_3$ at room temperature and atmospheric pressure to give **12** as oil (67% yield): $[\alpha]^{22}_D$ (b) −144.8° (*c* 0.59, $CHCl_3$); MS, m/z 296 (M^+); ^1H NMR ($CDCl_3$) δ 0.90 (d, J = 5.6, 3 H, CH_3 at C-10), 0.97 (d, J = 7.3, 3 H, CH_3 at C-11), 1.21 (t, J = 7.1, 3 H, CH_2CH_3), 152 (s, 3 H, CH_3 at C-4), 2.40 (m, 1 H, H-11), 3.43 (m, 1 H, CH_2CH_3), 3.92 (m, 1 H, CH_2CH_3), 4.43 (d, J = 6.1, 1 H, H-12), 5.29 (s, 1 H, H-5); IR (b) (film) ν_{max} 2950, 1450, 1380, 1200, 1050, 1000 cm^{-1}. Anal. (b) ($C_{17}H_{28}O_4$) C, H.

Biology. A. In Vitro Assessment of Antimalarial Activity. The intrinsic antimalarial activities of arteether and various control drugs were quantitatively assessed by using modifications[31] of the semiautomated microdilution method of Desjardins.[32] Drugs were 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

(29) A total of 800 g of QHS (**1**) was converted into arteether (**6**) in four separate batches.

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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 harvesting 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 fifty percent inhibitory concentrations were calculated for each drug (Table III).

Two control *Plasmodium falciparum* clones derived by direct visualization and micromanipulation[33] were utilized.

B. Blood Schizontocidal Activity in Mice. The activity of the α-DQHS ethyl ether was compared with that of arteether and QHS in mice infected with a drug-sensitive strain of *Plasmodium berghei* and a range of drug-resistant lines of this parasite and *P. yoelii*.[27] The compounds were dissolved in water or suspended by sonication with 10 mL of and 0.1% solution of Tween 80. Animals received a range of doses as a single, subcutaneous dose of drug on each of 4 consecutive days, starting 3 h after infection with parasitised donor blood.[34] Parasites were counted in thin blood films made from each animal and from sham-treated controls 1 day after the end of treatment. Activity was assessed by comparing the levels of parasitaemia in treated animals with those in the controls. The 50% and 90% effective levels (ED_{50}, ED_{90}) were estimated from log dose/probit-activity graphs. An index of resistance at the ED_{90} level was also calculated.[34]

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Registry No. 1, 63968-64-9; 2, 112346-66-4; 2-Na, 112419-28-0; 3, 71939-50-9; 4, 71963-77-4; 5, 71939-51-0; 6, 75887-54-6; 7, 82534-75-6; 8, 72826-63-2; 9a, 94668-17-4; 9b, 112419-27-9; 10, 112297-78-6; 11, 112297-79-7; 12, 112346-67-5.

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Synthesis and Anticandidal Properties of Polyoxin L Analogues Containing α-Amino Fatty Acids

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Analogues of polyoxin L containing amino acids with saturated fatty acid like side chains were synthesized from the benzyloxycarbonyl-protected α-amino fatty acid *p*-nitophenyl ester and uracil polyoxin C. Transfer hydrogenolysis using palladium black and formic acid gave diastereomeric, dipeptidyl polyoxin L analogues containing α-aminooctanoic acid (**3**), α-aminododecanoic acid (**4**), or α-aminohexadecanoic acid (**5**) as the amine terminal residue in 40–60% yield. Diastereomers of **3** and **5** were resolved by using high-performance liquid chromatography on a reversed-phase column and designated as **3a**, **3b** and **5a**, **5b**. Analogues **3–5** were excellent inhibitors of chitin synthetase from *Candida albicans*; **4**, the best inhibitor, had an ID_{50} of 0.5 μM. The L,L diastereomers of **3** and **5** were 1–2 orders of magnitude more potent chitin synthetase inhibitors than their D,L homologues. None of the synthetic polyoxin L analogues inhibited transport of trimethionine, but **3a**, **4**, and **5b** caused decreases of 71%, 87%, and 83%, respectively, in the initial rate of uptake of dileucine. Compounds **3–5** were significantly more stable to peptidase degradation than polyoxin L analogues containing naturally occurring α-amino acids. Compound **4** inhibited growth of *C. albicans* in culture at 40–80 μg/mL. All other analogues were less potent antifungals. The results suggest that synthetic polyoxins can be designed to have increased affinity for a peptide transport system and to have increased stability against intracellular degradation in *C. albicans*.

Polyoxins[1] and nikkomycins[2-4] (neopolyoxins[5-7]) are peptidyl nucleoside antibiotics that exhibit marked activity against phytopathogenic fungi. They inhibit the enzyme chitin synthetase,[1,8,9] which catalyzes the biosynthesis of chitin, and bear a structural resemblance to uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc), the natural substrate of this enzyme. Our laboratory has demonstrated that polyoxin D, one of the most active natural polyoxins, is toxic to the zoopathogenic fungi *Candida albicans* and *Cryptococcus neoformans* at millimolar concentrations.[10] The large number of infections caused by *C. albicans* in compromised hosts has fueled a major campaign by medicinal chemists to discover a more effective anticandidal drug. One approach to this problem involves the synthesis of novel polyoxin derivatives that will ultimately find application in the clinical treatment of patients with fungal infections.

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