dissolved in an ethanol/water mixture (1:1, volume), by iv or id injection. Plasma samples were obtained by cardiac puncture at 1 h after iv administration. Urine and feces were collected, from other rats, for 24 h after iv or id drug administration. Prior to radioassay, the feces and carcass were homogenized separately in 70% aqueous ethanol. Samples were assayed for total radioactivity by liquid-scintillation spectrometry and were corrected for tritiated water, as determined by lyophilization and radioassay of the distillates.

Plasma samples were lyophilized, and the residues were extracted with 50% aqueous ethanol or 20% aqueous acetonitrile. Metabolic patterns in clarified urine and fecal samples, and plasma extracts, were determined by thin-layer chromatography on silica gel GF plates (5 × 20 cm, 250-μm thick; Redi-Plates, Fisher Scientific Co.), which were developed in *n*-butanol/concentrated NH_4OH/water (20:1:1, by volume), *n*-butanol/chloroform/concentrated NH_4OH/water (20:5:1:1, by volume), or *n*-butanol/isopropyl alcohol/concentrated NH_4OH/water (10:7:2:4, by volume). Sections of the silica gel were sequentially scraped from the plates and radioassayed by liquid-scintillation counting. The radioactive zones were tentatively identified by comparison of their R_f values with those of authentic reference compounds, which were visualized by exposure of the plates to iodine vapors or spraying with ninhydrin.

Registry No. **3**, 98760-08-8; **4**, 107202-61-9; (2 *R*, 3 *S*)-**5a**, 110205-74-8; (2 *S*, 3 *S*)-**5a**, 110205-75-9; (2 *R*, 3 *S*)-**6a**, 110205-76-0; (2 *S*, 3 *S*)-**6a**, 110206-05-8; (2 *S*, 3 *S*)-**6b**, 110206-06-9; (2 *R*, 3 *S*)-**6b**, 110206-07-0; **7**, 110269-24-4; **7a**, 110206-08-1; **7a** (N-deblocked, HCl salt), 110206-10-5; **7b**, 110206-09-2; **8**, 110269-25-5; **9**, 110205-77-1; **10**, 110269-26-6; **11**, 110205-78-2; **12**, 110205-79-3; **13**, 110205-80-6; **14**, 110205-81-7; **15**, 110269-27-7; **16**, 110205-82-8; **17**, 110205-83-9; **18**, 110205-84-0; **19**, 110205-85-1; **20**, 110205-86-2; **21**, 110269-28-8; **22**, 110205-87-3; **23**, 110205-88-4; **24**, 110205-89-5; **25**, 110205-90-8; **26**, 110205-91-9; **27**, 110205-92-0; **28**, 110205-93-1; **29**, 110269-29-9; **30**, 110205-94-2; **31**, 110205-95-3; **32**, 110205-96-4; **33**, 110205-97-5; **34**, 110205-98-6; **34** (*N*-deblocked, 2 HCl salt), 110206-11-6; **35**, 110205-99-7; **36**, 110269-30-2; **37**, 110206-00-3; **38**, 110206-01-4; **39**, 110269-31-3; **40**, 110206-02-5; **41**, 110269-32-4; **42**, 110311-40-5; **43**, 110206-03-6; **44**, 110206-04-7; BOC-His-OH, 17791-52-5; c-$C_6H_{11}SH$, 1569-69-3; *i*-PrSH, 75-33-2; $PhOCH_2COCl$, 701-99-5; renin, 9015-94-5.

Notes

3-Pyrroline *N*-Oxide Bis(carbamate) Tumor Inhibitors as Analogues of Indicine *N*-Oxide

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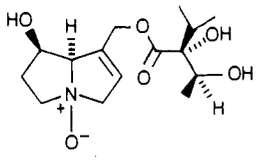
The 2,3-bis[[(*N*-methylcarbamoyl)oxy]methyl]-3-pyrroline 1-oxide **5** was synthesized and tested in the murine P388 lymphocytic leukemia model. The compound showed significant reproducible activity and was more potent than indicine *N*-oxide. 1-Methyl-2-phenyl-3,4-bis[[(*N*-2-propylcarbamoyl)oxy]methyl]-3-pyrroline *N*-oxide (**6**) was less active than **5**, and the 5,5-dimethyl analogue of **6**, the pyrroline *N*-oxide **7**, was inactive. The *N*-oxide **7** cannot be converted to a pyrrole in vivo because of the *gem*-dimethyl substitution at C-5.

Indicine *N*-oxide (NSC 132319), **1**, an alkaloid isolated from *Heliotropium indicum*, underwent human clinical trials as an antineoplastic agent.[1-4] The compound is a



1

pyrrolizidine alkaloid *N*-oxide but lacks the significant hepatotoxicity[5-15] associated with many of the pyrrolizidine alkaloid free bases, **2**. Some of the free bases do possess antineoplastic activity,[16-19] but this is overshadowed by hepatotoxicity. The toxicity and antineoplastic activity

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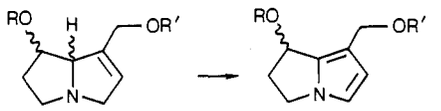
of the free bases are associated with the reactive pyrrole metabolites **3** derived from the alkaloids.[5-15]



2 **3**

In the case of indicine *N*-oxide two major questions arise. First, what is the mechanism of action of this agent and second, why does it lack the significant hepatotoxicity associated with the pyrrolizidine alkaloid free bases? The mechanism of action of indicine *N*-oxide is not known. It does not have significant electrophilic reactivity[20,21] so it is unlikely to function as an alkylating or acylating agent *without some prior metabolic activation*. It could function as an antimitotic agent or perhaps as an inhibitor of some metabolic pathway, with or without prior metabolic transformation, but such speculations remain unconfirmed.

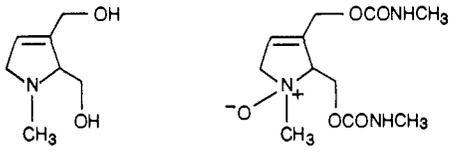
Myelosuppression is the dose-limiting toxicity of indicine *N*-oxide when the drug is given intravenously.[1-3] When the drug is given orally, it has hepatotoxicity comparable to that of indicine.[22-24] The hepatotoxicity of *oral* indicine *N*-oxide has been attributed to the reduction of the *N*-oxide by the intestinal flora to give indicine, which is subsequently converted to the reactive pyrrole in the liver.[22-24] The contrast between the hepatotoxicity of oral vs. intravenous indicine *N*-oxide invites the conclusion that indicine *N*-oxide is not converted to indicine, at least in significant amounts, when given intravenously. This conclusion is supported by several studies on the metabolism of indicine *N*-oxide.[22-29]

In one study of human cancer patients, 40% of the indicine *N*-oxide administered by intravenous infusion was excreted unchanged in the urine in 24 h and 2% was excreted as the free base indicine.[22] The fact that a large quantity of administered indicine *N*-oxide is recovered unchanged does not exclude the possibility that the drug acts as a prodrug, and in particular, reduction of the *N*-oxide in a hypoxic tumor cell presents the interesting possibility of indicine generation in the tumor. Indicine *N*-oxide is given in very high doses and it is possible that a small fraction of the dose could be converted to an active metabolite. If indicine *N*-oxide is being converted to an active metabolite and if only a small fraction of indicine *N*-oxide is converted to that metabolite, then variations in the amount of active metabolite produced from patient to patient would be responsible for the unpredictable antineoplastic and toxic effects of the drug that are observed.

Several indicine *N*-oxide analogues have been reported in which the esterifying "necic acid" portion has been varied.[30] The results of these studies showed that changes in potency and toxicity resulted from changes in the esterifying "necic acid" moiety. We report a series of monocyclic 3-pyrroline analogues of indicine *N*-oxide. The 2,3-dihydroxy-2-(2-propyl)butanoate moiety of **1** was replaced by an *N*-methylcarbamate moiety which, like the hindered carboxylic acid ester, is more resistant to hydrolysis in vivo. The carbamate moiety can also act as a hydrogen-bond donor and/or acceptor like the α-hydroxy ester of **1**.

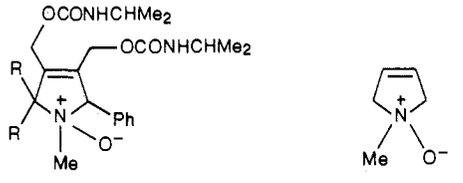
Chemistry

The monocyclic indicine *N*-oxide analogue with the α,β-substitution pattern was prepared from the diol **4**. The diol **4** was prepared by the procedure described by Mattocks.[31] Thus, sarcosine was treated with diethyl fumarate to give diethyl *N*-[(ethoxycarbonyl)methyl]-2-(methylamino)succinate (68%) that was cyclized in a Dieckmann condensation to give diethyl 1-methyl-4-oxopyrrolidine-2,3-dicarboxylate (67%). Reduction of the ketone (sodium borohydride in ethanol at −5 °C) gave diethyl 4-hydroxy-1-methylpyrrolidine-2,3-dicarboxylate (32%), which was converted to the tosylate and heated in pyridine to give diethyl 1-methyl-3-pyrroline-2,3-dicarboxylate (42%). Reduction of the diester (diisobutylaluminum hydride) gave the diol **4** (40%). Treatment of **4** with methyl isocyanate gave the bis(carbamate) (79%) that was oxidized (hydrogen peroxide–methanol) to give the *N*-oxide **5** (48%).



4 **5**

Two additional β,β'-substituted pyrroline *N*-oxides were synthesized to determine the effect of ring substitution on the activity of the 3-pyrroline *N*-oxides. The bis(carbamate) *N*-oxides **6** and **7** were prepared by *m*-chloroperbenzoic acid oxidation of the corresponding 3-pyrroline bis(carbamate).[32] The unsubstituted *N*-oxide **8** was prepared from 3-pyrroline.[33]



6: R = H
7: R = Me

8

Results and Discussion

The *N*-oxides were tested against murine P388 lymphocytic leukemia in vivo and the results are summarized in Table I. The monocyclic *N*-oxide **5** shows comparable activity to and is more potent than indicine *N*-oxide. The optimum dose for **5** is 100 mg/kg whereas the optimum dose for indicine *N*-oxide[30] is 800–1600 mg/kg. The phenyl-substituted pyrroline *N*-oxide **6** was less active than **5**, while the *gem*-dimethyl-substituted compound **7** and the

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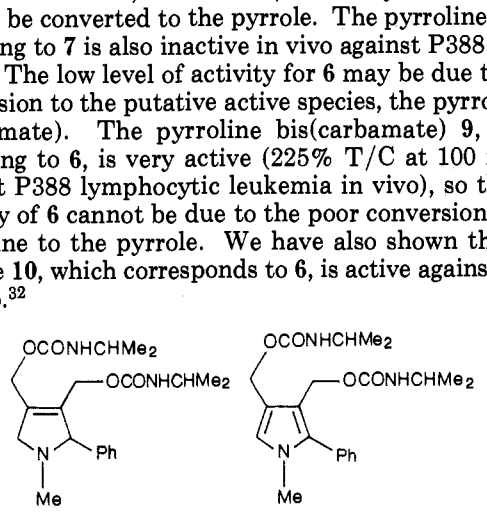
Table I. Antileukemic Activity (P388) of Pyrrolizine N-Oxides 5–8, Pyrroline 9, and Pyrrole 10[a]

compd	dose,[b] mg/kg	KE[c]	% T/C[d]	wt change:[e] T - C, g
5	400	0.34	143	-5.7
	200	1.00	150	-2.4
	100	1.70	159	-2.1
	50	0.62	145	-1.5
	25	-1.23	122	-1.2
	12.5	0.39	142	-3.8
6	100	-0.11	134	-1.5
	50	-1.16	116	-0.6
	25	-1.50	109	-0.1
	12.5	-1.21	115	1.6
7	100	-1.53	107	0.7
	50	-1.53	107	0.9
	25	-1.64	100	0.1
	12.5	-1.54	108	2.1
8	240	-0.12	108	-0.2
9	100	5.18	225	-2.2
	50	0.53	145	-2.0
	25	0.00	136	-0.9
	12.5	0.84	121	0.0
10[f]	30	1.99	171	-3.4
	15	2.63	181	-3.2
	7.5	0.32	145	-1.9
	3.75	-0.70	129	-0.5

[a] Determined under the auspices of the National Cancer Institute. For general screening procedures and data interpretation, see: Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3*, **1972**, 1; also see NIH Publication No. 84-2635, "In Vivo Cancer Models, 1976–1982". [b] The test drug given by intraperitoneal injection of a solution in distilled water. Five daily doses were administered beginning 24 h after tumor inoculation (ip). No test animal deaths were observed on or before day 5 of the test. [c] KE refers to the log cell kill, it is the log of the number of tumor cells at the end of treatment relative to the size at the beginning of treatment. The larger the value of KE, the greater the cell kill achieved. [d] % T/C is the ratio, expressed as a percent, of the survival of treated compared to untreated (control animals). [e] Wt change refers to the weight change of treated relative to control animals. [f] Suspensions of this compound were prepared fresh daily and used immediately following preparation.

simple pyrroline N-oxide **8** were inactive.

The inactivity of **7** may be due to the fact that this pyrroline N-oxide, with the 5,5-dimethyl substitution, cannot be converted to the pyrrole. The pyrroline corresponding to **7** is also inactive in vivo against P388 leukemia.[32] The low level of activity for **6** may be due to poor conversion to the putative active species, the pyrrole bis(carbamate). The pyrroline bis(carbamate) **9**, corresponding to **6**, is very active (225% T/C at 100 mg/kg against P388 lymphocytic leukemia in vivo), so the low activity of **6** cannot be due to the poor conversion of the pyrroline to the pyrrole. We have also shown that the pyrrole **10**, which corresponds to **6**, is active against P388 in vivo.[32]



9 **10**

The data presented in this report are consistent with the hypothesis that the N-oxides require prior conversion to the pyrrole in order to express antineoplastic activity. A comparison of the activities of **6**, **9**, and **10** reveal, however, that the pyrroline N-oxide prodrug modification of **10** is not an effective way to mask this reactive compound even though the N-oxides are much more water soluble and stable than the corresponding pyrrole. It remains a possibility that the N-oxide **6** could be a more efficient progenitor for **10** in a hypoxic tumor cell model.

Experimental Section

Melting points (uncorrected) were determined in open capillary tubes on a Thomas-Hoover Unimelt apparatus. IR spectra were determined with a Nicolet FT-IR and NMR spectra were determined for deuteriochloroform solutions containing 1% TMS on a Varian FT-80 NMR spectrometer. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA, and were within ±0.4 of the calculated values.

2,3-Bis-[[(*N*-methylcarbamoyl)oxy]methyl]-1-methyl-3-pyrroline 1-Oxide (5). A mixture of **4** (3.5 g, 24 mmol) in methyl isocyanate (20 mL) was stirred at 25 °C under nitrogen. Diazabicyclo[2.2.2]octane (10 mg, 0.8 mmol) was added and the solution stirred at reflux for 1.5 h. The excess methyl isocyanate was removed in vacuo and the residue was dissolved in dilute HCl (50 mL). The aqueous solution was washed with chloroform (3 × 25 mL) and made alkaline with ammonium hydroxide. The alkaline solution was extracted with chloroform (5 × 25 mL) and the chloroform extract was dried (sodium sulfate). The solvent was removed in vacuo to give an oil that was chromatographed (alumina, 9:1 acetone/methanol) to give the bis(carbamate) as a pale yellow oil (4.9 g, 79%): ^{1}H NMR δ 2.5 (s, 3 H), 2.76 (d, J = 4.5 Hz, 3 H), 2.83 (s, 3 H), 3.50 (m, 4 H), 4.13 (t, 1 H, J = 4.5 Hz), 4.63 (s, 2 H), 5.25 (br s, 2 H, D_2O exchangeable), 5.76 (br s, 1 H); IR (neat) 3343, 2944, 1710, 1535, 1421, 1329, 1269, 1145 cm^{-1}.

Hydrogen peroxide (15 mL, 30% H_2O_2) was added to a solution of the bis(carbamate) (4.5 g, 17.5 mmol) in methanol (75 mL) and the mixture was stirred at reflux for 1.5 days. The solution was cooled (ice bath) and manganese dioxide (activated brown) was added slowly until a negative potassium–starch iodide test was obtained. The reaction mixture was filtered over a bed of Celite, and the solvent was removed in vacuo to give a yellow oil. Chromatography (alumina, 8:2 acetone/methanol to 100% methanol) gave an oil that was dissolved in dry acetonitrile (50 mL) and the solvent removed in vacuo to give **5** as a white foam (2.3 g, 48%): mp 95–100 °C dec; ^{1}H NMR δ 2.63 (s, 3 H), 3.23 (s, 3 H), 4.20 (br), 4.36 (br), 4.63 (br), (7 H for all three broad signals), 5.93 (s, 1 H), 7.37 (br s, 2 H); IR (KBr) 3400, 1710, 1540, 1420, 1258, 1138, 985 cm^{-1}; ^{13}C NMR ($CDCl_3$–TMS) δ 27.83, 54.62, 60.72, 61.17, 70.41, 78.15, 124.46, 136.03, 157.41, 157.91. Anal. ($C_{11}H_{19}N_3$) C, H, N.

1-Methyl-2-phenyl-3,4-bis[[(*N*-2-propylcarbamoyl)oxy]methyl]-3-pyrroline 1-Oxide (6). A solution of 1-methyl-2-phenyl-3,4-bis[[(*N*-2-propylcarbamoyl)oxy]methyl]-3-pyrroline[32] (**9**; 3.5 g, 8.9 mmol), *m*-chloroperoxybenzoic acid (2.32 g of 80% pure, 13 mmol), and dichloromethane (125 mL) was stirred at 25 °C for 24 h. The solvent was evaporated under reduced pressure to yield a yellow gum that was chromatographed (silica gel eluted with 95:5 ethyl acetate–methanol) to give **6** as a pale yellow foam (2.05 g, 54%): ^{1}H NMR δ 1.13 (m, 12 H), 2.79 (s, 3 H), 4.50 (complex m, 11 H), 7.36 (s, 5 H); ^{13}C NMR δ 22.92, 43.18, 43.43, 55.41, 58.27, 58.72, 77.44, 95.04, 128.25, 129.48, 129.62, 130.65, 155.01, 155.54; IR (KBr) 3340, 3323, 3182, 2971, 1710, 1534, 1456, 1252, 1076 cm^{-1}. Anal. ($C_{21}H_{31}N_3O_5 \cdot H_2O$) C, H, N.

1,2,2-Trimethyl-5-phenyl-3,4-bis[[(*N*-2-propylcarbamoyl)oxy]methyl]-3-pyrroline 1-Oxide (7). A solution of 1,2,2-trimethyl-5-phenyl-3,4-bis[[(*N*-2-propylcarbamoyl)oxy]methyl]-3-pyrroline[32] (7.0 g, 17 mmol), *m*-chloroperoxybenzoic acid (4.34 g of 80% pure, 25 mmol), and dichloromethane (125 mL) was stirred at 25 °C for 2 days and then filtered, and the filtrate was concentrated in vacuo to yield a yellow oil that was chromatographed (silica gel eluted with 7:2:1 methanol–dichloromethane–ethyl acetate) to give a yellow foam that was chromatographed (basic alumina eluted with methanol) to give **7** as a pale yellow foam (6.29 g, 80%): mp 75–80 °C dec; ^{1}H NMR δ 1.23 (m, 18 H), 2.66 (s, 3 H), 3.43 (s, 3 H, methanol), 3.83 (quint, 2 H, J = 6 Hz), 5.00 (complex m, 7 H), 7.43 (s, 5 H); IR (KBr) 3318, 3165, 2978, 1703, 1533, 1249, 1076, 919 cm^{-1}. Anal. ($C_{23}H_{35}N_3O_5 \cdot$MeOH) C, H, N.

1-Methyl-3-pyrroline 1-Oxide (8). Hydrogen peroxide (7.02 g, 50% aqueous solution, 210 mmol) was added to a solution of

1-methyl-3-pyrroline[33] (5.73 g, 70 mmol) and methanol (50 mL) at 0 °C. The solution was stirred at 0 °C for 0.5 h, 25 °C for 0.5 h, and at reflux for 15 h. The reaction mixture was cooled to 0 °C and manganese dioxide was added slowly until a negative potassium–starch iodide test was obtained. The solvent was removed under reduced pressure to give a brown oil that was chromatographed (basic alumina eluted with 95:5 methanol–ammonium hydroxide) to give an oil which was decolorized (charcoal), yielding 8 as a yellow oil (4.3 g, 63%): ^{1}H NMR δ 3.23 (s, 3 H), 4.26 (m, 4 H), 5.86 (s, 2 H); IR (neat) 3224, 1442, 1358, 1027, 970, 914 cm^{-1}. Anal. ($C_5H_9NO\cdot0.65H_2O$) C, H, N.

Acknowledgment. This research was supported by Grant No. RO1-CA-22935, awarded by the National Cancer Institute, DHHS.

Registry No. 4, 53365-59-6; 5, 109719-73-5; 6, 109719-74-6; 7, 109719-75-7; 8, 109719-76-8; 9, 104156-41-4; 2,3-bis[[(*N*-methylcarbamoyl)oxy]methyl]-1-methyl-3-pyrroline, 57432-53-8; 1,2,2-trimethyl-5-phenyl-3,4-bis[[(*N*-2-propylcarbamoyl)oxy]-methyl]3-pyrroline, 104156-47-0; 1-methyl-3-pyrroline, 554-15-4.

Antimalarial Activity of New Water-Soluble Dihydroartemisinin Derivatives[1]

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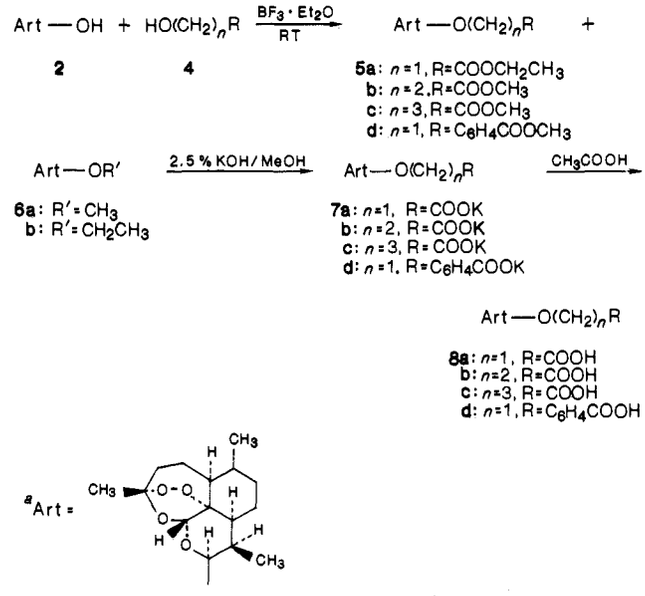
The usefulness of sodium artesunate (3), a water-soluble derivative of artemisinin (1), is impaired by its poor stability in aqueous solution. To overcome the ease of hydrolysis of the ester group in 3, a new series of derivatives of dihydroartemisinin (2) was prepared in which the solubilizing moiety, which contains a carboxylate group, is joined to dihydroartemisinin by an ether rather than an ester linkage. The new derivatives were prepared in good yield by treatment of dihydroartemisinin with an appropriate alcohol under boron trifluoride etherate catalysis at room temperature. All major condensation products are the β isomer. Hydrolysis of the esters with 2.5% KOH/MeOH gave the corresponding potassium salts, which were converted to free acids (**8b–d**) by acidification. The derivatives were tested in vitro against two clones of human malaria, *Plasmodium falciparum* D-6 (Sierra Leone clone) and W-2 (Indochina clone). No cross-resistance to the antimalarial agents mefloquine, chloroquine, pyrimethamine, sulfadoxine, and quinine was observed. In general, the new compounds are more effective against the W-2 than the D-6 strain. Esters (**5a–d**) possess activity comparable to that of the parent compounds 1 and 2; however, conversion of the esters to their corresponding carboxylates (**7a–d**) or acids (**8b–d**), with the exception of artelinic acid (**8d**), drastically decreases the antimalarial activities in both cell lines. Artelinic acid, which is both soluble and stable in 2.5% K_2CO_3 solution, possesses superior in vivo activity against *Plasmodium berghei* than artemisinin or artesunic acid.

Artemisinin (*qinghaosu*, 1), a clinically useful antimalarial agent that was isolated from the plant *Artemisia annua*, is an unusual sesquiterpene lactone containing an epidioxide function.[2-8] Dihydroartemisinin (2), obtained by sodium borohydride reduction of 1, was reported[9] to be more therapeutically active than the parent compound.

Scheme I[a]



Neither 1 nor 2 exhibit cross-resistance to chloroquine and both were proven efficacious against cerebral malaria in humans.[6] Sodium artesunate (3), the salt of the succinic acid half-ester derivative of dihydroartemisinin, is water soluble and can be administered by intravenous injection. This makes the compound particularly useful in the

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