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Biomimetic Metabolism of Artelinic Acid by Chemical Cytochrome P-450 Model Systems

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Purpose. To study the reaction of artelinic acid with chemical model systems of cytochrome P-450 as a means of obtaining authentic samples of the putative metabolites necessary for identification of the mammalian metabolites of artelinic acid.

Methods. Artelinic acid was reacted with different organic complexes of iron(II). The reaction products were isolated and characterized by NMR and thermospray mass spectroscopy.

Results. Five compounds which are putative metabolites of artelinic acid were isolated from these reactions and unambiguously identified, while the identity of two other compounds await final confirmation.

Conclusions. Standards of possible metabolites of artelinic acid can be produced by the reaction of the compound with ferrous complexes that may simulate cytochrome P-450 catalyzed metabolism of xenobiotics. This approach may provide a simple and versatile method for the formation of metabolites of artemisinin compounds which is more advantageous than previous approaches with fungal-based systems.

KEY WORDS: antimalarial; artelinic acid; metabolites; cnemical simulation of metabolism.

INTRODUCTION

Qinghaosu (QHS) (also known as artemisinin), a sesquiterpene lactone with an endoperoxide linkage, is the novel, clinically active antimalarial principle isolated from the Chinese medicinal herb, Qinghao or *Artemisia annua* L (1,2).

The development of more potent semi-synthetic derivatives of QHS has focused on the reduction of QHS to the lactol, dihydroqinghaosu (or dihydroartemisinin; Figure 1, DQHS), and subsequent preparation of its ether (or ester) derivatives.

Artelinic acid (Figure 1, compound [**1**]), the 4-carboxybenzyl ether of DQHS, was developed in this institute (3). A study of the *in vitro* metabolism of artelinic acid has been initiated, towards the goal of developing artelinic acid as an oral treatment for uncomplicated malaria caused by multidrug resistant *P. falciparum*.

The major obstacle in metabolism studies of artemisinin analogues is the non-availability of standards for putative metabolites. This problem arises because total synthesis of the metabolites involves tedious and laborious steps. So far the only known approach to making authentic standards of the metabolites of an artemisinin analogue is through fermentation of the compound with fungi (4–8). However, a limitation of the fungal fermentation method is that it is difficult to predict a microorganism that will closely mimic the mammalian metabolism of the compound. Reported data have also shown that fungal metabolism of these compounds does not correlate well with the mammalian metabolism. (4).

We have recently sought a more efficient and less time-consuming method for making the metabolites of the artemisinin compounds through the reaction of the compounds with chemical model systems of cytochrome P-450. The present work describes the biomimetic metabolism of artelinic acid by chemical models of cytochrome P-450 and the isolation and identification of putative metabolites of artelinic acid.

MATERIALS AND METHODS

Chemicals

All reagents and chemicals were obtained from Aldrich Chemical Co (Milwakee, USA) except artelinic acid and sodium artelinate which were from the inventory of the Walter Reed Army Institute of Research, Division of Experimental Therapeutics.

Instrumentation

Isolation of Reaction Products

Preparative HPLC was performed on a Waters liquid chromatography system consisting of two Waters model 510 solvent delivery units, a U6K injector and a Waters model 440 UV detector set at 254 nm. A Waters μ Bondapak C_{18} preparative column (7.8 mm × 300 mm; 10 mm) was used with a mobile phase consisting of 0.1M ammonium acetate buffer (pH 4.5) and acetonitrile. Separation was done using either a stepwise gradient of 70:30 (v/v) 0.1M ammonium acetate:acetonitrile maintained for 56 min, then changed to 50:50, and held at this ratio for a further 40 min (flow rate 2.0 ml/min); or a gradient of 80:20 (v/v) 0.1M ammonium acetate:acetonitrile maintained for 40 min, changed to 70:30 (v/v) for 46 min, and then stepped to 50:50 and held for a further 30 min. Acetonitrile was removed from the collected fractions under a stream of nitrogen and the fractions were extracted with ethyl acetate (3 ml) by shaking on a vortex mixer for 2 min. The ethyl acetate extracts were dried over anhydrous sodium sulphate and evaporated under a stream of nitrogen. The residues were crystallized from a mixture of diethyl ether and hexane. The isolated products were again re-analysed by HPLC-MS as described below, to establish their purity.

The reaction products may also be separated by repeated preparative thin layer chromatography on 10 × 20 cm preparative silica gel GF plates, with dichloromethane:ethyl acetate (50/50, v/v) as solvent. Detection was by UV light, with the compounds showing up as dark bands on the fluorescent background. The bands were separated and extracted with 20% methanol in ethyl acetate, the solvent removed, and the residue crystallized from ether-hexane.

Characterization of Reaction Products

Mass spectrometric identification of the reaction products was performed using a HPLC-MS system consisting of a Hew-

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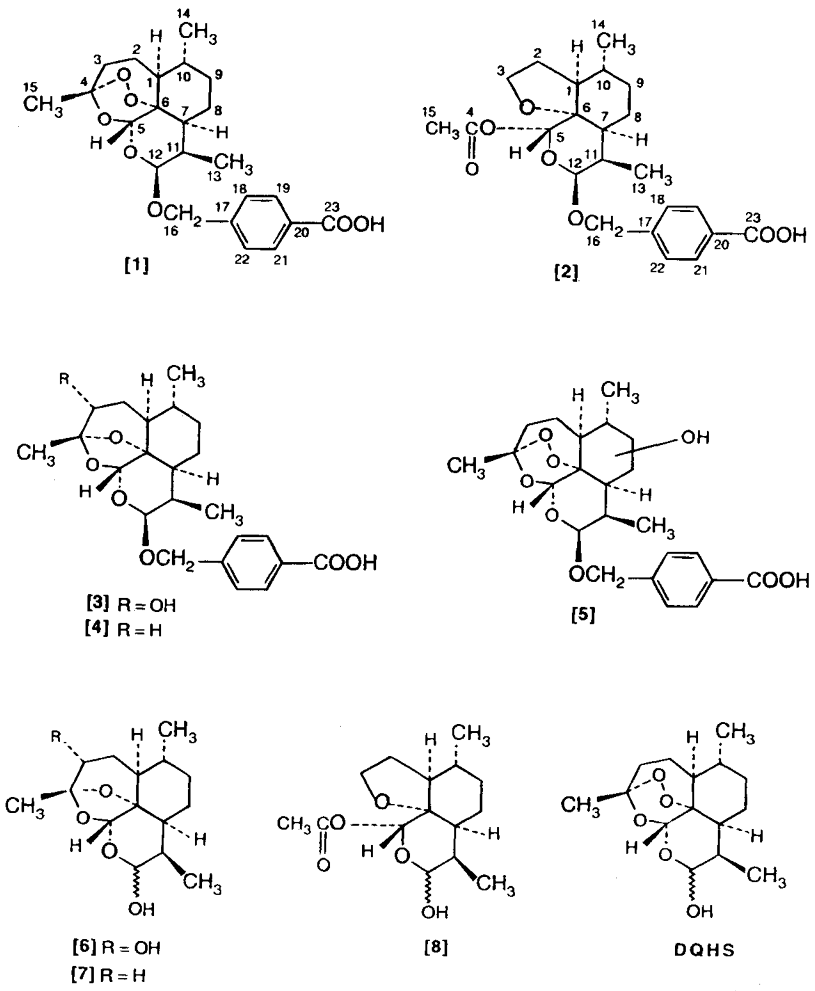

Fig. 1. Chemical structures of artelinic acid and analogues.

lett Packard 1090 Liquid Chromatograph System linked with a HP 5989A MS engine via a Hewlett Packard thermospray interface. A μBondapak, C_{18} column (2.1 mm × 100 mm; 5 m) was used, with a mobile phase consisting of 0.1M ammonium acetate (pH 4.5) and acetonitrile. Elution was done with either of two linear gradients: 95:5 (v/v) 0.1M ammonium acetate:acetonitrile maintained for 10 min and then ramped to 30:70 at 60 min, and held for 20 min (flow rate 1.0 ml/min), or 90:10 (v/v) 0.1M ammonium acetate:acetonitrile maintained for 10 min, then increased to 60:40 at 60 min, and held at this ratio for 30 min (flow rate 1.0 ml/min). The thermospray interface was operated in the "fragmenter on" mode, with the thermspray probe tip temperature maintained at 220–230°C throughout the run, and a source temperature of 226°C.

^{1}H- and ^{13}C-nmr spectra were obtained using a Brucker AC 300 Spectrometer, using $CDCl_3$ as solvent. Chemical shifts (δ, ppm) and coupling constants (J, Hz) were measured relative to Me_4Si.

Melting points (uncorrected) were determined on a Thomas Hoover Unimelt^R capillary melting point apparatus.

Reaction of Artelinic Acid with the Udenfriend System (Fe^{2+}-Ascorbic Acid-O_2)

Ferrous sulphate heptahydrate (1.22 g; 4.39 mmole) was dissolved in 100 ml of water followed by disodium-EDTA dihydrate (3.25 g; 8.73 mmole). After stirring the mixture for about 15 min, sodium artelinate hydrate (1 g; 2.18 mmole) was added followed by ascorbic acid (15.38 g; 87.33 mmole). The mixture was stirred vigorously for 15 h on magnetic stirrer, and then extracted twice with 60 ml of ethyl acetate, by stirring for 1 h. The extracts were combined and the ethyl acetate removed with a stream of nitrogen to give an oil. which was fractionated by repeated preparative TLC or by preparative HPLC.

Reaction of Artelinic Acid with Hemin and Thiol-containing Compunds

Hemin (780 mg; 1.2 mmol) was dissolved in 50 ml of acetone. While stirring vigorously with a magnetic stirrer, a solution of 300 mg (2.5 mmol) of cysteine in 10 ml of water was added, followed by 250 mg (0.6 mmol) of artelinic acid. The mixture was stirred at room temperature for 18 h. The acetone was removed with a stream of nitrogen. The residue was stirred with 100 ml of water for 20 min and centrifuged at 3000 rpm for 15 min. The supernatant was extracted with 2 × 40 ml of ethyl acetate for 30 min and the precipitate extracted with 4 × 30 ml of diethyl ether by shaking on a vortex mixer for 3 min. After evaporating the solvents both the ethyl acetate and the ether extracts yielded a dark brown oil, with more of the material being obtained from the ether extract of the precipitate. For initial purification, the oil was chromatographed on preparative TLC plates with ethyl acetate:dichloromethane (30:70, v/v) as solvent. A resinous material remained at the origin and the main band (rf about 0.3) was scraped and extracted with a 20:80 (v/v) methanol:ethyl acetate mixture yielding a pale yellow solid. The solid was fractionated by preparative HPLC.

The above procedure was repeated, using 740 mg (2.4 mmol) of glutathione or 500 mg (3.24 mmol) of thiosalicylic acid instead of cysteine.

Reaction of Artelinic Acid with Iron(II) Sulphate and Thiosalicylic Acid

Iron(II) sulphate (831.4 mg; 2.99 mmol) was dissolved in 50 ml of water and a solution of 922.2 mg (5.98 mmol) of thiosalicylic acid in 50 ml of acetone was added followed by 500 mg (1.2 mmol) of artelinic acid The mixture was stirred at room temperature for 18 h, during which time a beige-colored precipitate appeared. The mixture was centrifuged (3000 rpm, 10 min) and the supernatant (pH 3) was extracted with 50 ml of ethyl acetate. The extract was dried over anhydrous sodium sulphate and evaporated to obtain an oil. The oil was purified by preparative TLC. Three bands were formed of which the middle band (5.5–10.6 cm) was the main product. The material in the main band was recovered and purified by preparative HPLC.

Reaction of Artelinic Acid with Iron(II) Chloride and 8-Hydroxyquinoline (Oxine)

Iron(II) chloride tetrahydrate (475.6 mg: 2.392 mmol) was dissolved in about 10 ml of water and a solution of 694.5 mg (4.78 mmol) of 8-hydroxyquinoline (oxine) in 10 ml of acetone was added. A dark blue mixture formed immediately. Artelinic acid (200 mg; 0.479 mmol) was added and the mixture stirred for 16 h. The mixture was centrifuged (3000 rpm for 15 min), the supernatant separated and dried with a stream of nitrogen. The resulting residue was extracted with 40 ml of ethyl acetate. The ethyl acetate extract was washed successively with 2 × 20 ml of 0.05 M hydrochloric acid, 20 ml of distilled water, dried over anhydrous sodium sulphate, filtered through fluted filter paper and evaporated to dryness. The oily residue obtained was purified by preparative HPLC.

RESULTS AND DISCUSSION

Identification and Characterization of Compounds Formed

Extracts of reaction mixtures were initially examined by LC-MS to establish the presence of new compounds before chromatographic fractionation of the extracts was attempted. The total ion chromatograms of the crude products of the reaction of artelinic acid with the different reagents are shown in Figures 2 to 5. Chromatograms obtained for the products of the reaction of artelinic acid with hemin and thiosalicylic acid are also similar to those in Figure 3, except for the absence of peak [**5**].

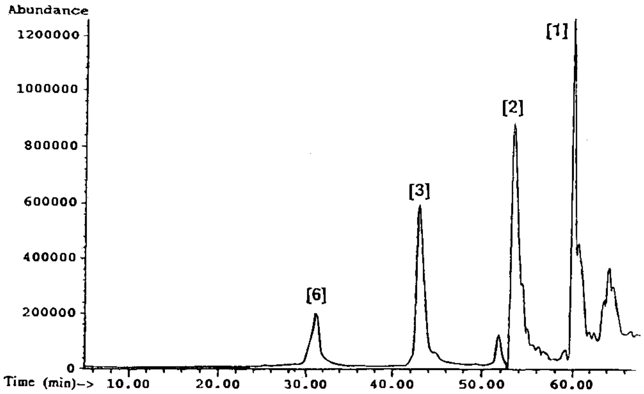

Fig. 2. Total ion chromatogram of the crude products of the reaction of artelinic acid with the Udenfriend system.

The structures of the five compounds isolated and characterized after the reaction of artelinic acid with the above model systems of cytochrome P-450 are shown in Figure 1. The identity of two other compounds (Figure 1, [**5**] and [**8**]) suspected to be formed by these reaction requires final confirmation. Of the compounds identified and characterized, the previously unreported ones are rearranged artelinic acid [**2**], 3α-hydroxy deoxyartelinic acid [**3**], and deoxy artelinic acid [**4**]. The ^{1}H- and ^{13}C-nmr assignments for these compounds are based on the published NMR data for analogous compounds obtained by the microbial metabolism of arteether (6,8) and artemisinin thermodecomposition products (9).

The relevant nmr and mass spectra data for artelinic acid (Figure 1, [**1**]) are provided for comparison: ^{13}C-nmr, see Table I. ^{1}H-nmr: 0.95 (3H, d, J = 6.0, Me-14), 0.98 (3H, d, J = 7.4, Me-13), 1.46 (3H, s, Me-15), 2.72 (1H, m, H-11), 2.03 (1H, m, H-3β), 2.40 (1H, d, d, d, J = 3.9, 4.07, 3.9, H-3α), 2.72 (1H, m, H-11), 4.61 (1H, d, J = 13.3, H-16β), 4.94 (1H, d, J = 3.4, H-12), 4.99 (1H, d, J = 13.3, H-16α), 5.47 (1H, s, H-5), 7.43 (2H, d, J = 8.2, H-18, H-22), 8.10 (2H, d, J = 8.2, H-19, H-21). Thermospray mass spectrum (m/z, relative intensity (%): 436, 3.2%; 401, 5.2%; 373, 4.0%; 359, 3.1%; 355, 3.0%; 331, 15.2%; 284, 3.1%; 249, 3.8%; 221, 100%.) In all the mass spectra presented, the highest m/z values are for the quasi-molecular $[M + NH_4]^+$ ion. The thermospray mass spectra of artelinic acid and related compounds formed in these reactions are characterized by fragmentation of the carboxybenzyl group (as carboxybenzyl alcohol, $OHCH_2C_6H_4COOH$; 152

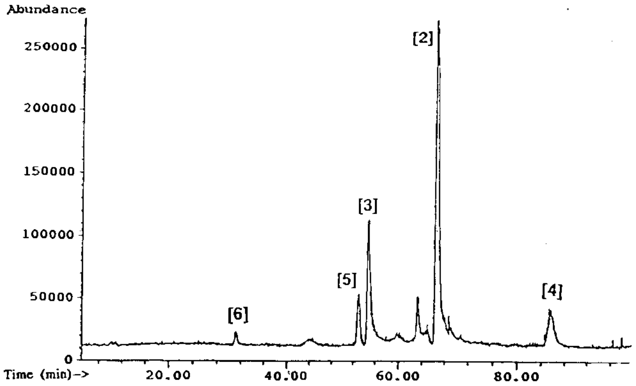

Fig. 3. Total ion chromatogram of the crude products of the reaction of artelinic acid with hemin-cysteine.

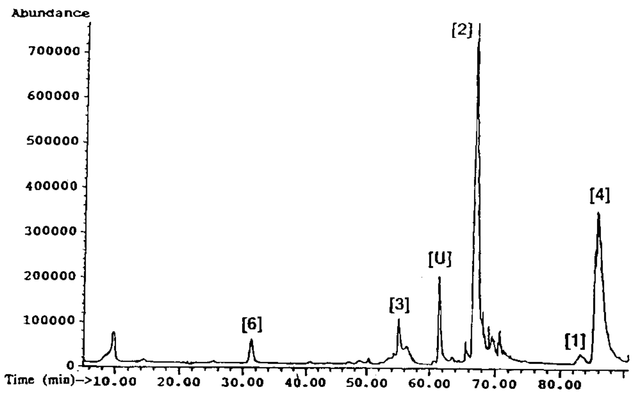

Fig. 4. Total ion chromatogram of the crude products of the reaction of artelinic acid with Iron(II)-thiosalicylic acid.

amu) giving rise to spectra with low abundance of the molecular or quasi-molecular ammonium adduct ions. The occurrence of the m/z 359 ion in the mass spectrum of artelinic acid, attributable to the loss of CH_3COOH from the protonated molecular ($[M + H]^+$-60), as found in the mass spectrum of the rearranged compound [**2**] (see below), suggests that artelinic acid undergoes thermally-induced rearrangement to [**2**] during mass spectrometry, a phenomenon also observed with the thermodecomposition of artemisinin (9).

Rearranged artelinic acid (Figure 1, [**2**]) melting point 186–188°. ^{13}C-nmr, see Table I. ^{1}H-nmr: 0.93 (3H, d, J = 7.5, Me-14), 0.96 (3H, d, J = 7.8, Me-13), 2.17 (3H, s, Me-15), 2.47 (1H, m, H-11), 3.95 (1H, q, J = 8.0, 8.4, 7.7, H-3β), 4.28 (1H, d, bs, d, J = 2.2, 2.1, H-3α), 4.52 (1H, d, J = 13.0, H-16β), 4.84 (1H, d, J = 4.1, H-12), 5.05 (1H, d, J = 13.0, H-16α), 6.32 (1H, s, H-5), 7.46 (2H, d, J = 8.1, H-18, H-22), 8.09 (2H, d, J = 8.1, H-19, H-21). Thermospray mass spectrum (m/z, relative intensity (%): 436, 0.4%; 401, 1.2%; 376, 7.0%; 359, 18.2%; 331, 11%; 284, 0.2%; 242, 1.7%; 225, 16.0%; 207, 7.2%; 179, 6.3%.) This compound is analogous to rearranged arteether (designated AEM1) which was found to be a microbial metabolite of arteether (5). Apart from features in the thermospray mass spectrum due to loss of the carboxybenzyloxy side-chain, the thermospray mass spectrum of the rearranged compound [**2**] is also characterized by the M-60 peak ($[M + NH_4]^+$-CH_3COOH) resulting from theu fragmentation of the acetate group.

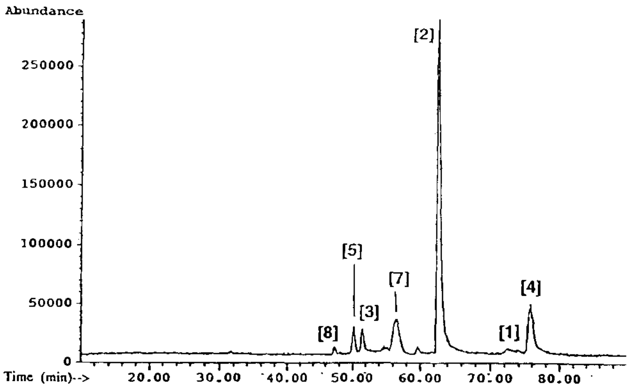

Fig. 5. Total ion chromatogram of the crude products of the reaction of artelinic acid with Iron(II)-oxine.

Table I. ^{13}C-NMR Chemical Shift Assignments for Compounds 1–4

Carbon No.	Chemical shift assignment (ppm)			
	[1]	[2]	[3]	[4]
1	52.4	55.5	40.5	46.6
2	24.6	27.6	30.3	24.3
3	36.3	68.5	69.5	34.7
4	104.1	169.3	107.9	108.0
5	87.9	88.3	93.6	94.6
6	81.0	80.4	84.1	83.9
7	44.2	46.7	42.4	40.8
8	24.4	24.7	25.0	22.0
9	43.5	35.7	34.7	34.4
10	37.3	30.5	34.6	35.1
11	30.8	33.3	30.2	30.4
12	101.6	101.2	99.5	99.0
13	13.0	12.4	12.4	12.3
14	20.2	20.4	18.7	18.9
15	26.0	21.5	20.9	25.0
16	69.1	68.9	69.2	69.1
17	130.2	130.0	130.2	130.1
18	126.8	127.3	126.9	126.9
19	128.2	127.3	126.2	126.9
20	144.5	144.4	144.5	144.8
21	128.2	127.3	126.9	126.9
22	126.8	127.3	126.9	126.9
23	171.3	170.7	170.9	170.8

The compound [**2**] was found to be a major product of the reaction of artelinic acid with all the model systems examined. The isolated yield of [**2**] ranged from 4 to 6%, with the hemin-containing systems giving yields of 15 to 20 mg of [**2**]. We have also obtained preliminary results on the metabolism of artelinic acid by human liver microsomes showing [**2**] to be one of the major human metabolites of artelinic acid.

3α-Hydroxydeoxyartelinic acid (Figure 1, [**3**]) melting point 128–130°. ^{13}C-nmr, see Table I. ^{1}H-nmr: δ 0.88 (3H, d, J = 6.3, Me-14), 1.00 (3H, d, J = 7.4, Me-13), 1.55 (3H, s, Me-15) 2.52 (1H, m, H-11), 3.58 (1H, q, H-3), 4.59 (1H, d, J = 13.6, H-16β), 4.86 (1H, d, J = 4.2, H-12), 4.92 (1H, d, J = 13.6, H-16α), 5.29 (1H, s, H-5), 7.41 (2H, d, J = 8.08, H-18, H-22), 8.07 (2H, d, J = 8.04, H-19, H-21). Thermospray mass spectrum (m/z, relative intensity (%): 436, 0.7%; 401, 1.7%; 285, 4.3%; 284, 28.1%; 267, 16.7%; 266, 8.5%; 249, 34.4%; 239, 4.0%; 231, 4.6%; 222, 14.3%, 221, 100%.) 3α-Hydroxydeoxyartelinic acid is analogous to 3α-hydroxydeoxyarteether, a known microbial and human metabolite of arteether. Isolated yields of 7 to 12 mg (percentage yield 3 to 5%) of 3α-hydroxydeoxyartelinic acid were obtained from the reactions. Preliminary studies have also shown 3α-hydroxydeoxyartelinic acid to be an *in vitro* metabolite of artelinic acid in human liver microsomes. Identification of metabolites of artelinic acid in human liver microsomes will be reported as a separate paper.

Deoxyartelinic acid (Figure 1, [**4**]), melting point 116–118°. ^{13}C-nmr, see Table I. ^{1}H-nmr: δ 0.89 (3H, d, J = 5.8, Me-14), 1.00 (3H, d, J = 7.4, Me-13), 1.49 (3H, s, Me-15), 2.50 (1H, m, H-11), 4.59 (1H, d, J = 13.4, H-16β), 4.89 (1H, d, J = 4.63, H-12), 4.94 (1H, bs, H-16α), 5.33 (1H, s, H-5), 7.43 (2H, d, J = 8.2 H-18, H-22), 8.07 (2H, d, J = 8.2, H-19, H-21).Thermospray mass spectrum (m/z, relative intensity (%):

420, 0.4%; 357, 1.1%; 268, 16.5%; 252, 16.7%; 251, 100%; 233, 45.5%; 223, 2.8%; 215, 22.4%; 205, 19.6%.) The ^{1}H-nmr and ^{13}C-nmr spectra of compound **[4]** are also consistent with those of deoxy arteether (5). The best yield of 23 mg (percent yield, 9%) of deoxyartelinic acid was obtained from the reaction system containing hemin and glutathione.

A hydroxyartelinic acid (Figure 1, **[5]**), with the position and stereochemistry of the hydroxy group as yet to be confirmed, is suspected to be one of the products of the reaction of artelinic acid with the hemin-based reagents and with the iron(II)-oxine reagent. The ^{1}H-nmr spectrum of this compound exhibits a quadruplet centered at δ 3.55 (5,6,8). The ^{1}H-nmr spectrum also displayed the same aromatic proton resonances as artelinic acid, indicating that the hydroxy group was not introduced onto the aromatic ring of artelinic acid. Thermospray mass spectrum (m/z, relative intensity (%): 452, 0.1%; 435, 0.2%; 392, 1.6%; 375, 3.4%; 357, 2.4%; 300, 35.7%; 283, 9.5%; 265, 18.1%; 240, 22.1%; 237, 13.5%; 224, 14.1%; 223, 100%; 222, 34.1%; 221, 10.1%; 195, 2.7%; 179, 7.3%; 177, 13.0%.) The thermospray mass spectrum is readily rationalized as that of a hydroxylated artelinic, with the formation of the ions involving the fragmentation of the 4-carboxybenzyl side-chain and thermal rearrangement of the compound during mass spectrometry, as experienced for artelinic acid itself.

3α-Hydroxydeoxydihydroqinghaosu (3α-OH-deoxy DQHS) (Figure 1, **[6]**) formed from artelinic acid was identified by comparison of its HPLC retention time, ^{1}H-nmr and mass spectra with those of an authentic standard. Thermospray mass spectrum (m/z, relative intensity (%): 302, 7.3%; 285, 11%; 284, 52.1%; 267, 52.9%; 266, 26.1%; 249, 100%; 239, 52.9%; 231, 17.9%; 221, 5.8%; 207, 6.8%.). 3α-OH-deoxyDQHS is a putative metabolite of artelinic acid and has also been reported to be a microbial metabolite of arteether (5).

Deoxydihydroqinghaosu (deoxyDQHS) (Figure 1, **[7]**) also was identified by comparison of its HPLC retention time and mass spectrum with those of an authentic standard. Thermospray mass spectrum (m/z, relative intensity (%): 286, 3.2%; 268, 16.2%; 252, 16.8%; 251, 100%; 233, 35.0%; 223, 59.9%; 215, 2.5%; 205, 3.3%.)

Rearranged dihydroqinghaosu (Figure 1, **[8]**) is suspected to be one of the compounds formed by the respective reaction of artelinic acid with the Udenfriend and the iron(II)-oxine systems on the basis of the similarity of its thermospray mass spectrum to that of rearranged artelinic acid. Thermospray mass spectrum of rearranged dihydroqinghaosu (m/z, relative intensity (%): 302, 4.4%; 284, 1.6%; 267, 2.0%; 242, 74.7%; 225, 19.3%; 224, 13.1%; 207, 17.2%; 206, 100%; 195, 0.8%.)

LC-MS of the extract of the Udenfriend system showed that DQHS was formed in this system.

4-(Hydroxymethyl)benzoic acid identified by its ^{1}H-nmr spectrum, was recovered by TLC from the reaction of artelinic acid with the Udenfriend, iron(II)-thiosalicylic and iron(II)-oxine systems.

No compound resulting from the hydroxylation of the aromatic ring of artelinic acid was observed in this study.

Simulation of the Metabolism of Artelinic Acid

Although many chemical models of P-450 have been studied, only very simple compounds such as cyclohexane, styrene, adamantane, aniline, toluidine, etc have been used as substrates. There have been only a few reports on the application of chemical models of the P-450 enzymes to study the metabolism of actual drugs. We studied the chemical simulation of the metabolism of artelinic acid as a means of obtaining milligram quantities of the likely metabolites of the compound.

From previous reports on the metabolism of artemisinin and arteether (4–8), the expected P-450 catalyzed metabolic reactions of artelinic acid are rearrangement and/or deoxygenation of the endoperoxide group, C-hydroxylation at the 1- or 2- or 3- or 9- or 14-position, and removal of the 4-carboxybenzyl side-chain.

The earliest of the chemical model systems of cytochrome P-450, which has come to be known as the Udenfriend system, consists of a mixture of ferrous ion, ascorbic acid, EDTA, and molecular oxygen (10). The Udenfriend system brought about the biomimetic rearrangement of artelinic acid to compound **[2]** and 3α-hydroxydeoxyartelinic acid **[3]**. This system also causes the removal of the 4-carboxybenzyl side-chain of artelinic acid. Deoxygenation of artelinic acid to deoxyartelinic acid was not observed with this reagent.

The simulation of cytochrome P-450 by the combination of hemin and thiol-containing compounds such as cysteine and glutathione was extensively studied by Sakurai and co-workers (11). The present work is the first attempt to apply these systems to a study of the metabolism of a drug. When the reaction of artelinic acid and the hemin-thiol reagents was carried out in a purely aqueous medium most of the artelinic acid was recovered unchanged, with only traces of **[2]** and deoxyartelinic acid **[4]** being detected by LC-MS. When the reaction was carried out in aqueous acetone, a virtually complete transformation of artelinic acid was observed, with the unchanged compound being only detectable by LC-MS. The hemin-thiol systems brought about the deoxygenation of artelinic acid to deoxyartelinic acid. These systems were virtually ineffective in the dealkylation of artelinic acid. Thus, neither DQHS nor deoxyDQHS was formed, and only traces of 3α-OH-deoxyDQHS were detected, in the hemin-thiol systems. A compound suspected to be a hydroxyartelinic acid was also formed in the hemin-thiol systems.

The combination of iron(II) with thiosalicylic acid is one of the early variations on the Udenfriend system, and this reagent was found to hydroxylate simple aromatic and aliphatic hydrocarbons (12). The iron(II)-thiosalicylic acid system brings about the biomimetic rearrangement, deoxygenation and dealkylation of artelinic acid. Also, a compound (Figure 4, **[U]**) suspected to be a hydroxylated rearranged artelinic acid was detected by LC-MS from the iron(II) thiosalicylic acid system.

The simulation of cytochrome P-450 drug metabolizing activity by the combination of iron(II) and 8-hydroxyquinoline (oxine) has not been reported previously. The iron(II)-oxine system caused the rearrangement of artelinic acid into **[2]** and 3α-hydroxydeoxyartelinic acid **[3]**, catalyzed its deoxygenation to deoxyartelinic acid **[4]** and possible hydroxylation to a hydroxyartelinic acid. In addition, this system is capable of effecting biomimetic dealkylation as evidenced in the formation of deoxyDQHS and the compound tentatively identified as rearranged DQHS.

CONCLUSIONS

Standards of metabolites of artelinic acid can be produced by the reaction of the compound with simple reaction systems which simulate cytochrome P-450 catalyzed drug metabolism. This approach is simple and rapid, and is more advantageous than the reported approach to the formation of the metabolites of artemisinin compounds based on their fermentation with fungi.

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