Microbial Transformation of Artemether

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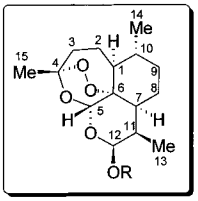
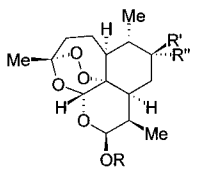
Microbial transformation of the semisynthetic sesquiterpene artemether, using *Cunninghamella elegans* and *Streptomyces lavendulae*, resulted in the isolation of five microbial metabolites that were characterized on the basis of their ^{1}H and ^{13}C NMR spectral data. These metabolites were identified as 9β-hydroxyartemether, 9α-hydroxyartemether, ring-rearranged 9β-hydroxyartemether, 3α-hydroxydeoxyartemether, and 14-hydroxyartemether. The latter three compounds are new and are being reported here for the first time.

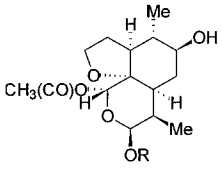
The vicious reemergence of malaria constitutes a major problem in many tropical regions, especially subsaharan Africa, where 1−2 million patients perish every year. What worsens the situation is the emergence of *Plasmodium* strains which are resistant to the currently few available drugs, mainly, quinine, chloroquine, and sulfadoxine−pyrimethamine.[1] Such a situation has called for an active search for novel antimalarial compounds from natural, semisynthetic, or synthetic routes. Compounds originating from the antimalarial Chinese herb *Artemisia annua* (Asteraceae) are promising new natural product lead compounds. Dihydroartemisinin is the reduction product of artemisinin, the main active sesquiterpene constituent of *A. annua*.[2] Earlier studies showed that the ethyl ether derivative of dihydroartemisinin, arteether (**1**), is a promising candidate for use with high-risk malaria patients including those with cerebral malaria.[3] In the course of clinical development of arteether, metabolism studies were conducted utilizing mammalian as well as microbial systems.[4−7] Another related compound is artemether (**2**), the methyl ether derivative of dihydroartemisinin.[3]



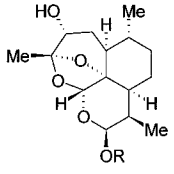
(1) R = CH_3CH_2
(2) R = CH_3



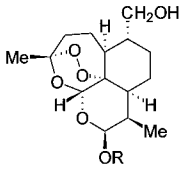
(6) R = CH_3CH_2, R' = OH, R'' = H
(3) R = CH_3, R' = OH, R'' = H
(11) R = CH_3CH_2, R' = H, R'' = OH
(9) R = CH_3, R' = H, R'' = OH



(7) R = CH_3CH_2
(4) R = CH_3



(8) R = H
(5) R = CH_3



(12) R = CH_3CH_2
(10) R = CH_3

Since artemether was also found to be more effective *in vivo* than artemisinin, probably due to its better lipophilicity and chemical stability of the trioxane system,[3] its clinical efficacy was tested intramuscularly in malaria patients and proven to be very effective and well tolerated in moderately severe *Plasmodium falciparum* infections.[8−10] As a matter of fact, artemether has lately become a renewed hope for combating the emerging generations of resistant malaria in underdeveloped countries. In view of the highly promising clinical results, a microbial transformation study was conducted by our group in order to characterize the major artemether metabolites which are likely to be very similar to the mammalian ones. This work may contribute to future development of artemether by providing some new analogs that may serve as prospective candidates for antimalrial evaluation or as starting compounds for the semisynthesis of various artemether derivatives.

Results and Discussion

Twenty-five cultures were screened for their ability to biotransform **2**. All were able to completely or partially convert **2** to more polar metabolites. Of these, *Cunninghamella elegans* (ATCC 9245) and *Streptomyces lavendulae* (L-105) were chosen for preparative scale fermentations in order to compare their results with those previously obtained from arteether fermentations. *C. elegans* was capable of completely exhausting **2** and converting it into three more polar metabolites, **3**, **4**, and **5**. Absorption around 3400−3500 cm^{-1} in the IR spectra of these metabolites confirmed that they were alcohols. Metabolite **4** showed an absorption peak at 1755 cm^{-1}, indicating a carbonyl group and suggesting a rearranged metabolite. The MS of **3** and **4** showed molecular ions which were 16 mass units higher than that of **2**. The HRFABMS data were consistent with a molecular formula of $C_{16}H_{26}O_6$ for **3** and **4**. Comparison of the ^{13}C and ^{1}H NMR data of metabolites **3**−**5** with previously published data for the arteether metabolite analogs **6**−**8**[5,6] were in complete agreement. The proposed structure for **5** relies on comparative NMR data with the arteether metabolite **8**. On the basis of these data the metabolites were assigned as follows: metabolite **3** is 9β-hydroxyartemether, metabolite **4** is ring-rearranged 9β-hydroxyartemether, and metabolite **5** is 3α-hydroxydeoxyartemether.

S. lavendulae was also able to convert **2** into three more polar metabolites, **9**, **10**, and traces of a partially

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pure metabolite which showed correlation with the major metabolite **3** isolated from *C. elegans*. Again, the IR absorption of the three metabolites around 3400−3500 cm^{-1} confirmed their hydroxylation. No carbonyl group absorption was noticed in any of these metabolites. The MS of **9** and **10** showed molecular ions which were 16 mass units higher than that of **2**. The HR-FABMS data were consistent with a molecular formula of $C_{16}H_{26}O_6$ for the two metabolites. Comparison of the ^{13}C and ^1H NMR data of metabolites **9** and **10** with previously published data for the arteether metabolite analogs **11** and **12**[6] were in complete agreement. On the basis of these data the metabolites were assigned as follows: metabolite **9** is 9α-hydroxyartemether, and metabolite **10** is 14-hydroxyartemether. TLC and NMR data for the third impure metabolite suggested that it is **3**, the 9*β*-hydroxyartemether. Thus, *S. lavendulae* was able to convert artemether to its two 9-hydroxy isomers, with the the 9α-hydroxy isomer as the major metabolite. This observation, that *S. lavendulae* has the enzyme machinery for producing the 9α- and 9*β*-hydroxy isomers in artemisinin systems, has been previously reported.[5,11]

Of the five isolated metabolites, compounds **4**, **5**, and **10** are being reported for the first time.

Experimental Section

General Experimental Procedures. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The IR spectra were recorded with a Perkin-Elmer 281B infrared spectrophotometer. The ^{13}C and ^1H NMR spectra were obtained in $CDCl_3$ on a Varian VXR-300 FT spectrometer operating at 75 and 300 MHz, respectively. The chemical shift values are reported as ppm units, and the coupling constants are in Hz. Low-resolution MS were obtained using a thermospray Vestec Model 201 mass spectrometer operating in the filament-on mode. HRFABMS (Fisons/VG Autospec Q) was carried out at the University of Kansas. TLC analyses were carried out on precoated silica gel Si 250F plates (Baker). The developing system used was ethyl acetate−toluene (3:2) solution, and visualization of plates was performed using anisaldehyde−H_2SO_4 spray reagent.[12] For column chromatography, the adsorbent used was Si gel 230−400 mesh (Merck).

Metabolism and Organisms. Microbial metabolism studies were conducted as previously reported.[5] Twenty-five fungal cultures, obtained from the University of Mississippi, Department of Pharmacognosy culture collection, were screened and are listed below; the number of metabolites present on TLC is indicated in parentheses: *Acrodictys erecta* ATCC 24083 (0); *Aspergillus alliaceus* NRRL 315 (5); *Aspergillus flavus* NRRL 626 (3); *Aspergillus niger* ATCC 10549 (2); *Aspergillus tamarii* NRRL 8101 (0); *Beauvauria bassiana* ATCC 7159 (3); *C. elegans* ATCC 9245 (3); *Cunninghamella blakesleeana* UM-ATCC 8688a; *Lipomyces lipofer* ATCC 10742 (3); *Melanospora ornata* ATCC 26180 (4); *Mortierella zonata* ATCC 13309 (2); *Mucor ramannianus* 1839 (sih) (4); *Nocardia corallina* ATCC 19070 (3); *Nocardia minima* ATCC 19150 (4); *Penicillium chrysogenum* ATCC 9480 (3); *Penicillium patulum* ATCC 24550 (4); *Rhizopus stolonifer* ATCC 24795 (3); *Saccharomyces cerevisiae* ATCC 9763 (1); *Septomyxa affinis* ATCC 6737 (2); *Streptomyces griseus* NRRL 5687 (5); *S. lavendulae* L-105 (3); *Streptomyces rimosus* NRRL 2234 (5); *Streptomyces roseochromogens* ATCC 13400 (2); *Thamnidium elegans* ATCC 18191 (1); *Trichophyton mentagrophytes* ATCC 9972 (3).

Microbial Metabolism of Artemether (2) by *C. elegans*. *C. elegans* (ATCC 9245) was grown in 20 1-L culture flasks, each containing 250 mL of medium. A total of 1000 mg of **2** (in 9.2 mL of EtOH) was evenly distributed among the 24-h old stage II cultures. After 8 days, the incubation mixtures were pooled and filtered to remove the cells, and the filtrate (4.8 L) was extracted three times with EtOAc (1 × 4.8 L, 2 × 2.4 L). The combined extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness at 40 °C under reduced pressure to afford an orange brown residue. The residue (ca. 1100 mg) was purified by column chromatography over a silica gel column (100 g, 2.5 × 80 cm), using a hexane−ethyl acetate (1:1) mixture as an eluting system to yield 7 mg of **5** as a colorless oil and a single spot with $R_f = 0.60$, 134 mg of **3** with an $R_f = 0.57$ and 132 mg of **4** with an $R_f = 0.43$.

Compound **3**: colorless prisms (from hexane); mp 141−142 °C; IR ν_{max} (KBr) cm^{-1}: 3510 (−OH), 2990, 2960, 2930, 2880; HRFABMS *m/z* calcd for $C_{16}H_{27}O_6$ $[M + 1]^+$ 315.1808, found 315.1816; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.42 (1 H, s, H-5), 4.68 (1 H, d, $J = 3.3$ Hz, H-12), 3.41 (3 H, s, Me-16), 3.10 (1 H, ddd, $J = 11.0$, 9.5, 4.5 Hz, H-9), 2.60 (1 H, m, H-11), 2.36 (1 H, ddd, $J = 14.6$, 13.2, 4.0 Hz, H-3*β*), 1.9−2.1 (1 H, m, H-3α), 1.59 (1 H, m, H-7), 1.5−1.9 (2 H, m, H-2), 1.44 (3 H, s, Me-15), 1.2−1.4 (1 H, m, H-1), 1.05 (3 H, d, $J = 5.8$ Hz, Me-14), 0.90 (3 H, d, $J = 7.4$ Hz, Me-13); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 104.1 (s, C-4), 103.2 (d, C-12), 87.4 (d, C-5), 80.2 (s, C-6), 74.1 (d, C-9), 56.0 (q, C-16), 50.0 (d, C-1), 44.2 (d, C-10), 42.0 (d, C-7), 36.3 (t, C-3), 33.6 (t, C-8), 30.6 (d, C-11), 24.5 (t, C-2), 26.1 (q, C-15), 15.4 (q, C-14), 12.9 (q, C-13).

Compound **4**: colorless needles (from hexane); mp 188−189 °C; IR ν_{max} (KBr) cm^{-1} 3440 (−OH), 2965, 2905, 1755 (C=O); HRFABMS *m/z* calcd for $C_{16}H_{27}O_6$ $[M + 1]^+$ 315.1808, found 315.1817; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.32 (1 H, s, H-5), 4.61 (1 H, d, $J = 4.1$ Hz, H-12), 4.30 (1 H, ddd, $J = 8.4$, 4.4, 3.0 Hz, H-3a), 3.97 (1 H, ddd, $J = 8.0$, 8.0, 8.0 Hz, H-3b), 3.41 (3 H, s, Me-16), 3.22 (1 H, ddd, $J = 14.5$, 11.0, 5.8 Hz, H-9), 2.34 (1 H, m, H-11), 2.06−2.16 (2 H, m, H-8), 2.13 (3 H, s, Me-15), 1.71−2.02 (3 H, m, H-2 and H-7), 1.35−1.54 (2 H, m, H-1 and H-10), 1.04 (3 H, d, $J = 6.0$ Hz, Me-14), 0.88 (3 H, d, $J = 7.3$ Hz, Me-13); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.1 (s, C-4), 103.2 (d, C-12), 87.9 (d, C-5), 80.1 (s, C-6), 77.5 (d, C-9), 69.8 (t, C-3), 56.0 (q, C-16), 52.6 (d, C-1), 45.0 (d, C-7), 37.6 (d, C-10), 34.3 (t, C-8), 33.1 (d, C-11), 27.2 (t, C-2), 21.5 (q, C-15), 16.5 (q, C-14), 12.3 (q, C-13).

Compound **5**: colorless oil; ^1H and ^{13}C NMR data were in complete aggreement with data for the areteether analog **8**; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.26 (1 H, s, H-5), 4.64 (1 H, d, $J = 1.4$ Hz, H-12), 3.57 (1 H, dd, $J = 1.8$, 4.1 Hz, H-3), 3.39 (3 H, s, Me-16), 2.45 (1 H, m, H-11), 1.60−1.91 (6 H, m, H-1, H-2, H-8, and H-9a), 1.56 (3 H, s, Me-15), 1.34−1.45 (1 H, m, H-7), 1.20-1.30 (1 H, m, H-10), 0.90−0.99 (1 H, m, H-9b), 0.91 (3 H, d, $J = 7.4$ Hz, Me-13), 0.87 (3 H, d, $J = 6.3$ Hz, Me-14); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 107.9 (s, C-4), 101.5 (d, C-12), 93.5

(d, C-5), 84.2 (s, C-6), 69.6 (d, C-3), 56.0 (q, C-16), 42.5 (d, C-7), 40.7 (d, C-1), 34.8 (d, C-10), 34.7 (t, C-9), 30.4 (d, C-11), 30.3 (t, C-2), 25.0 (t, C-8), 21.0 (q, C-15), 18.8 (q, C-14), 12.3 (q, C-13).

Microbial Metabolism of Artemether (2) by *S. lavendulae*. *S. lavendulae* (L-105) was grown in 20 1-L culture flasks, each containing 250 mL of medium. A total of 1000 mg of **2** (in 9.2 mL of EtOH) was evenly distributed among the 24-h old stage II cultures. After 14 days, the incubation mixtures were pooled and filtered to remove the cells, and the filtrate (4.8 L) was extracted three times with EtOAc (1 × 4.8 L, 2 × 2.4 L). The combined extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness at 40 °C under reduced pressure to afford an orange brown residue. The residue (ca. 1300 mg) was purified by column chromatography over a silica gel column (100 g, 2.5 × 80 cm), using a toluene−ethyl acetate (4:1) mixture as an eluting system, to yield 115 mg of **9** with an $R_f = 0.60$, 7 mg of **10** with an $R_f = 0.50$, and 7 mg of impure **3** with an $R_f = 0.57$.

Compound **9**: colorless needles (from hexane); mp 134−135 °C; IR ν_{max} (KBr) cm^{-1}: 3535 (−OH), 2999, 2968, 2890; HRFABMS *m/z* calcd for $C_{16}H_{27}O_6$ $[M + 1]^+$ 315.1808, found 315.1830; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.39 (1 H, s, H-5), 4.69 (1 H, d, J = 3.5 Hz, H-12), 3.73 (1 H, ddd, J = 2.3, 2.3, 2.3 Hz, H-9), 3.42 (3 H, s, Me-16), 2.64 (1 H, m, H-11), 2.39 (1 H, ddd, J = 14, 14, 3.7 Hz, H-3α), 1.7−2.1 (5 H, m, H-1, H-2a, H-3β, H-7 and H-8), 1.44−1.55 (1 H, m, H-2b), 1.44 (3 H, s, Me-15), 1.03 (3 H, d, J = 6.7 Hz, Me-14), 0.89 (3 H, d, J = 7.4 Hz, Me-13); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 104.1 (s, C-4), 103.6 (d, C-12), 87.0 (d, C-5), 80.7 (s, C-6), 70.0 (d, C-9), 56.0 (q, C-16), 44.6 (d, C-1), 40.6 (d, C-10), 37.0 (d, C-7), 36.5 (t, C-3), 31.7 (t, C-8), 30.3 (d, C-11), 26.1 (q, C-15), 24.5 (t, C-2), 16.5 (q, C-14), 12.8 (q, C-13).

Compound **10**: mp 127−128 °C; IR ν_{max} (KBr) cm^{-1} 3422 (−OH), 2990, 2923, 2882; HRFABMS *m/z* calcd for $C_{16}H_{27}O_6$ $[M + 1]^+$ 315.1808, found 315.1816; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.39 (1 H, s, H-5), 4.69 (1 H, d, J = 3.2 Hz, H-12), 3.73 (1 H, dd, J = 10.7, 2.8 Hz, H-14a), 3.60 (1 H, dd, J = 10.7, 5.7 Hz, H-14b), 3.43 (3 H, s, Me-16), 2.63 (1 H, m, H-11), 2.40 (1 H, m, H-3a), 2.05 (1 H, m, H-3b), 1.7−1.9 (2 H, m, H-9), 1.5−2.1 (4 H, m, H-2 and H-8), 1.5−1.6 (1 H, m, H-1), 1.4−1.6 (2 H, m, H-7 and H-10), 1.45 (3 H, s, Me-15), 0.92 (3 H, d, J = 7.4 Hz, Me-13); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 104.0 (s, C-4), 103.4 (d, C-12), 87.7 (d, C-5), 81.2 (s, C-6), 65.0 (t, C-14), 56.0 (q, C-16), 47.0 (d, C-1), 44.4 (d, C-7 and C-10), 36.4 (t, C-3), 30.9 (d, C-11), 28.7 (t, C-9), 26.1 (q, C-15), 24.2 (t, C-2), 24.1 (t, C-8), 13.0 (q, C-13).

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