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STEREOSELECTIVE SYNTHESIS AND ANTIMALARIAL ACTIVITY OF α-ARTELINIC ACID FROM ARTEMISININ

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ABSTRACT.—α-Artelinic acid [**8**], a potent, stable, and water-soluble antimalarial agent, has been synthesized from artemisinin [**1**], the sesquiterpene lactone endoperoxide isolated from *Artemisia annua*. The blood schizontocidal antimalarial activity of α-artelinic acid evaluated against *Plasmodium knowlesi* is also reported.

Artemisinin [**1**] (qinghaosu, arteannuin), a unique sesquiterpene endoperoxide isolated from the Chinese medicinal plant *Artemisia annua* L. (Compositae), has been found to show remarkable blood schizontocidal activity against chloroquine-resistant and cerebral malaria with a fast action and remarkably few side effects (1,2). The cyclic hemiacetal dihydroartemisinin [**2**], obtained by $NaBH_4$ reduction of artemisinin, has been found to possess higher antimalarial activity than its parent natural product (2). The Chinese workers have prepared a number of ethers and esters of dihydroartemisinin to improve biological activity, stability, and bioavailability of the drug (3), and among the artemisinin derivatives prepared, the lipophilic compound arteether [**3**] and the H_2O-soluble sodium artesunate [**4**] have been studied clinically (4–6).

Sodium artesunate [**4**], which being H_2O-soluble could be administered iv, has been found particularly useful in the treatment of critical stages of cerebral malaria where rapid reversal of parasitemia and restoration of consciousness of the patient are of critical importance. However, the utility of artesunate [**4**] was impaired by its poor stability in aqueous solution due to the ease of hydrolysis of the hemiacetal ester group. In order to overcome the stability problem, Lin *et al.* (7) synthesized a series of H_2O-soluble and stable derivatives of dihydroartemisinin [**2**] in which solubilizing carboxylate groups were coupled to dihydroartemisinin by an ether rather than by a labile ester linkage. Among the derivatives prepared, the compound *p*-[(12β-dihydroartemisininoxy) methyl] benzoic acid [**5**], named artelinic acid, and its salt **6** showed excellent stability and in vivo antimalarial activity superior to that of artesunate [**4**] or artemisinin [**1**] against *Plasmodium berghei*. The artelinic acid was prepared by Lewis-acid mediated coupling of dihydroartemisinin [**2**] with methyl-*p*-(hydroxymethyl) benzoate followed by alkaline hydrolysis. The reaction sequence led to the preparation of the β-diastereoisomer (β-epimer) of artelinic acid [**5**].

In continuation of our work on the chemistry and drug development of artemisinin and its derivatives (8–11), we now report an efficient and stereoselective synthesis of α-artelinic acid [**8**], the opposite diastereoisomer of β-artelinic acid [**5**], as a new, stable, and H_2O-soluble compound which has shown excellent blood schizontocidal antimalarial activity.

Dihydroartemisinin [**2**] was prepared in 85% yield by $NaBH_4$ reduction of artemisinin [**1**] in MeOH at 0–5°. The reaction of **2** with methyl-*p*-(iodomethyl) benzoate in dry CH_2Cl_2 using freshly

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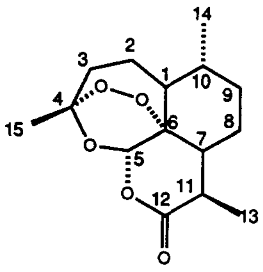
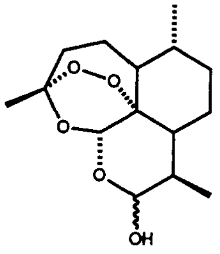


1



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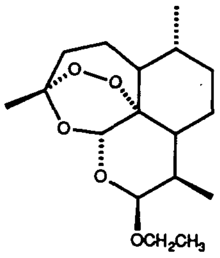

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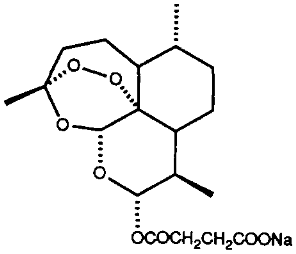

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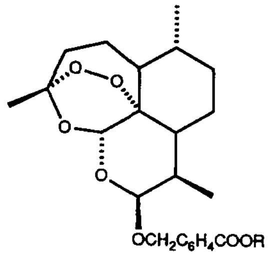
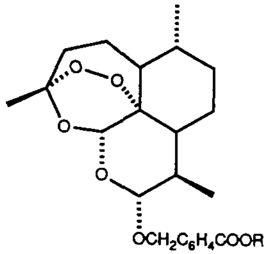


5 R=H
6 R=K



7 R=Me
8 R=H
9 R=Na

prepared Ag_2O at room temperature yielded exclusively the α-epimer **7** characterized as methyl-*p*-[(12α-dihydroartemisininoxy)methyl] benzoate. The stereochemistry at C-12 of **7** was assigned as α-equatorial by ^{1}H-nmr spectral data which showed a large coupling constant ($J=9$ Hz) between H-12 and H-11 as observed in the case of the α-diastereoisomeric arteether reported by us (8). Two magnetically non-equivalent benzylic methylene protons appeared as an AB quartet mainly due to close proximity of several chiral centers of the dihydroartemisinin moiety, and a large geminal coupling constant ($J=12$ Hz) between two benzylic protons was observed as reported in the case of β-artelinate (7). Therefore, compound **7** is a diastereoisomer or α-epimer of artelinate prepared by Lin *et al.* (7). Hydrolysis of **7**, 2.5% NaOH-MeOH at room temperature, afforded *p*-[(12α-dihydroartemisininoxy)methyl] benzoic acid [**8**] or α-artelinic acid. The sodium salt of α-artelinic acid was prepared by a reported method (7) and gave a white amorphous sodium-*p*-[(12α-dihydroartemisininoxy) methyl] benzoate [**9**], sodium α-artelinate.

α-Artelinic acid [**8**] dissolved in 5% $NaHCO_3$ solution was tested in rhesus monkeys infected with *Plasmodium knowlesi* at two different doses, 15 mg/kg × 3 days and 20 mg/kg × 3 days. Total parasite clearance from blood circulation was observed within 24 h after a first dose of 20 mg/kg. Out of two doses tested, a 20 mg/kg × 3 days dose was curative and no recrudescence was observed until 50 days of observation (treatment route was iv in aqueous solution). The α-artelinic acid [**8**] has been found to be very fast acting as shown by parasitemia clearance. Furthermore, it was safer, as the LD_{50} of **8** in Swiss mice was found to be 1000 mg/kg, a better therapeutic index than that of β-artelinate (7).

The new diastereoisomeric α-artelinic acid [**8**] is a fast acting, blood schizontocidal antimalarial agent potentially useful in the treatment of multi-drug-resistant and cerebral malaria caused by *Plasmodium falciparum*. Detailed preclinical pharmacological study of α-artelinate is currently being undertaken, and results will be published in due course.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Artemisinin [**1**] was isolated (14) from the aerial parts of *A. annua* cultivated by the Central Institute of Medicinal and Aromatic Plants, Lucknow in India (14), and dihydroartemisinin [**2**] was prepared by a reported procedure (12). The ^1H-nmr spectra were measured on a CFT-80 (Varian) spectrometer. The ir spectra were recorded on a 399 B Perkin-Elmer instrument, and the ms were determined on Finnigan MAT and Hitachi RMU 6L spectrometers. The sample of β-artelinate was generously given by Prof. D.L. Klayman of the Walter Reed Army Institute of Research, Washington, DC. Methyl-4-(iodomethyl) benzoate was prepared from *p*-toluic acid by a reported method (13).

METHYL-*p*-[(12α-DIHYDROARTEMISININOXY)METHYL] BENZOATE [**7**].—To a magnetically stirred suspension of dihydroartemisinin (2.84 g, 10 mmol), freshly prepared Ag_2O (2.31 g, 10 mmol) in dry CH_2Cl_2 (10 ml) and methyl-*p*-(iodomethyl) benzoate (2.76 g, 10 mmol) were added. The reaction mixture was stirred for 5 h and filtered, and the filtrate was washed with H_2O, dried over Na_2SO_4, and evaporated to afford diastereoisomerically pure methyl-*p*-[(12α-dihydroartemisininoxy) methyl] benzoate [**7**] as a viscous oily substance: yield 3.05 g (70%); ^1H nmr ($CDCl_3$) 7.95 (2H, d, $J=9$ Hz, H-3′ and H-7′), 7.35 (2H, d, $J=9$ Hz, H-4′ and H-6′), 5.19 (1H, s, H-5), 5.18 (1H , d, $J=12$ Hz, H-1′), 4.77 (1H, d, $J=12$ Hz, H-1′), 4.40 (1H, d, $J=9$ Hz, H-12), 3.85 (3H, s, -CO_2Me), 1.33 (3H, s, Me-15), 0.95 (3H, d, $J=7.2$ Hz, Me-13), 0.80 (3H, d, $J=3.6$ Hz, Me-14); ir ($CHCl_3$) 1720, 1620, 1440, 1330, 1280, 1105, 1020, 760 cm^{-1}. *Anal.* calcd for $C_{24}H_{32}O_7$, C 66.67, H 7.40; found C 67.2, H 7.00.

p-[(12α-DIHYDROARTEMISININOXY)METHYL] BENZOATE (α-ARTELINIC ACID) [**8**].—Compound **7** (4.32 g, 10 mmol) was dissolved in 2.5% NaOH/MeOH (35 ml) and kept at room temperature for 2 h. The solvent was removed in vacuo. The residue was dissolved in H_2O (40 ml), and the aqueous layer was neutralized with glacial HOAc and extracted with Et_2O. The Et_2O layer was washed with H_2O, dried over Na_2SO_4, and concentrated. An oily product (4 g) was obtained, which was purified by chromatography on Si gel: ^1H nmr ($CDCl_3$) 8.05 (2H, d, $J=8$ Hz, H-3′ and H-7′), 7.45 (2H, d, $J=8$ Hz, H-4′ and H-6′), 5.40 (1H, s, H-5), 4.97 (1H, d, $J=13.5$ Hz, H-1′), 4.75 (1H, d, $J=13.5$ Hz, H-1′), 4.50 (1H, d, $J=9.5$ Hz, H-12), 2.75 (1H, m), 1.47 (3H, s, Me-15), 1.05 (3H, d, $J=7.2$ Hz, Me-13), 0.90 (3H, d, $J=2.7$ Hz, Me-14). *Anal.* calcd for $C_{23}H_{30}O_7$, C 66.02, H 7.17; found C 65.5, H 7.57.

SODIUM-*p*-[(12α-DIHYDROARTEMISININOXY) METHYL]BENZOATE (SODIUM α-ARTELINATE) [**9**].—α-Artelinic acid [**8**] (1.09 g, 2.5 mmol) was dissolved in 2.5% NaOH/MeOH (4 ml) and allowed to stand at room temperature for 2 days. The solvent was reduced to half volume, diluted with an equal volume of H_2O, and washed with Et_2O to remove impurities. The aqueous solution was passed through reversed-phase Si gel (RP-18) using MeOH-H_2O (1:1) eluent, the MeOH was evaporated in vacuo, and the aqueous solution was lyophilized to give 0.65 g of **9** as a yellowish powder: mp 160° (dec). *Anal.* calcd for $C_{23}H_{29}O_7Na \cdot H_2O$, C 60.26, H 6.55; found C 61.3, H 5.95.

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