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EPI-DEOXYARTEANNUIN B AND 6,7-DEHYDROARTEMISINIC ACID FROM *ARTEMISIA ANNUA*

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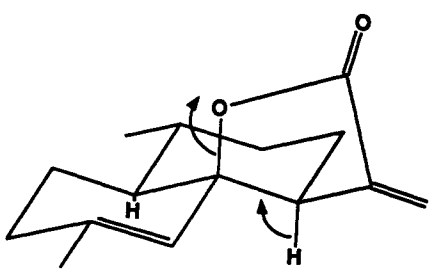
The Chinese herb, *Artemisia annua* L. (Compositae), contains the antimalarial compound artemisinin, a sesquiterpene lactone endoperoxide (1). Examination of the locally grown plant revealed the presence of two related constituents that were found to be *epi*-deoxyarteannuin B [**1**] and its elimination product, namely, 6,7-dehydroartemisinic acid [**2**]. This report describes the isolation and characterization of these compounds, including the conversion of **1** to **2**.

The physical and spectral properties of the less polar compound (R_f 0.87) were identical to those of *epi*-deoxyarteannuin B [**1**], recently reported (3) from the same source. It is interesting to note that this compound is slowly formed when an EtOH solution of artemisinic acid [**3**] is allowed to stand at room temperature for several days. Its formation as a by-product of singlet oxygen ($^1O_2$) oxidation of **3** has been previously reported (4). Thus, **1** appears to



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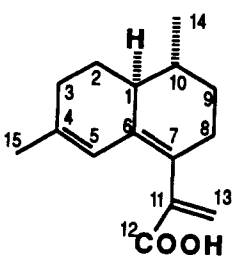

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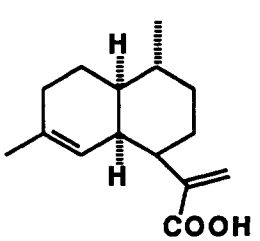
FIGURE 1. *Trans*-diaxial elimination of *epi*-deoxyarteannuin B [**1**] to 6,7-dehydroartemisinic acid [**2**] by heating with glacial HOAc at 100°.

The hexane extract of the leaves of *A. annua* was treated with MeCN as previously reported (1) in order to precipitate the waxes and other undesirable products. This was followed by partitioning between 10% aqueous MeOH and *n*-hexane. Tlc analysis of the hexane solubles on Si gel G plates, using Et_2O-hexane (4:3) as solvent, revealed the presence of a spot, R_f 0.87, running ahead of the spot due to artemisinic acid [**3**], and another spot tailing behind, R_f 0.37. Separation of these two compounds was accomplished by flash chromatography on Si gel (2) using the high solute-to-adsorbent ratio of 1:400.



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be, at least in part, an artifact derived from **3**. In our hands, **1** was also observed as a by-product of oxidizing **3** with numerous oxidizing agents including mercuric acetate, selenium dioxide, and chromium trioxide. In fact, it can be

cleanly prepared from **3** by oxidation with chromium chlorochromate, albeit in low yields.

The more polar compound (R_f 0.37), **2**, $C_{15}H_{20}O_2$, was obtained as colorless prisms, mp 129–130°, $[\alpha]_D$ +317° (c = 0.05, MeOH). Its nmr data (see Experimental) were similar to those of **3** (5), except for the absence of the two methines at C-6 and C-7. Instead, the ^{13}C-nmr spectrum revealed the presence of two olefinic singlets at δ 137.3 and 127.0. The structure and stereochemistry of **2** were unambiguously assigned by preparing it from *epi*-deoxyarteannuin B [**1**]. This was accomplished by heating it at 100° in glacial HOAc solution. Under these conditions, **1** underwent smooth *trans*-diaxial elimination to provide **3** in 75% yield, as shown in Figure 1. 6,7-Dehydroartemisinic acid [**2**] has not been previously reported either in *A. annua* or in other sources.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Ir spectra and specific rotations were obtained on Perkin-Elmer 58 OIR and 241 MC instruments, respectively. ^{1}H-nmr and ^{13}C-nmr spectra were determined on a Varian VSR-300 spectrometer at 300 and 75 MHz, respectively, and chemical shift values are given in δ (ppm) with TMS as internal standard. Standard pulse sequences were used for DEPTGL (6) and HETCOR (7) spectra, which aided nmr assignments. Low resolution electron impact mass spectra were obtained using an E.I. Finnigan model 3200 (70 eV ionization potential) with INCOS data system. Tlc was performed on Si gel G plates using Et_2O-hexane (4:3) as solvent, unless otherwise specified, and visualized under short wavelength uv light or by spraying with anisaldehyde spray reagent (8). *A. annua* was grown at the local medicinal plant garden. Greenhouse-grown plants were planted in early November 1986, and leaves were picked at the pre-flowering stage in early April 1987. A voucher specimen is preserved at the herbarium of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

ISOLATION OF 6,7-DEHYDROARTEMISINIC ACID FROM *A. ANNUA* LEAVES.—The leaves of *A. annua* (900 g) were continuously extracted with hexane in a Soxhlet apparatus for 3 days. The hexane extract (70 g) was treated with MeCN (200 ml), then filtered. Evaporation left 55 g of a dark residue that was partitioned between hexane (1200 ml) and 4 × 300 ml portions of 10% aqueous MeOH. The hexane solubles (30 g) were chromatographed (only 2.5 g was used) on a 55 × 4.5 cm column of Si gel using Et_2O-hexane (4:3) as the solvent system to give two main fractions, A and B.

Fraction A (0.222 g) contained primarily **1** and was further purified by flash chromatography (2) using C_6H_6-MeCO (99:1) as eluent to give 0.037 g of **1** as colorless oil that readily crystallized from *n*-hexane to give prisms, mp 81–82°, $[\alpha]_D$ +145° (c = 0.1, MeOH) [lit. (4) mp 81.5–83°, lit. (3) mp +142°] with ir, ^{1}H-nmr, and ^{13}C-nmr spectra identical to those of *epi*-deoxyarteannuin B. Fraction B (0.37 g) was a mixture of **2** (minor) and **3** (major) and was purified by repeated chromatography to give 0.028 g of **2** as colorless prisms from Et_2O/hexane, $C_{15}H_{20}O_2$, mp 129–130°, $[\alpha]_D$ +317° (c = 0.05, MeOH); ir (KBr) (cm^{-1}) 3200 (CO*OH*), 1678 (*C*OOH); uv (MeOH) (log ε) λ max 244 (4.21), 270 (3.28); ^{1}H nmr ($CDCl_3$) δ 9.46 (1H, br s, COO*H*, exchangeable), 6.46 (1H, d, J = 2.1 Hz, H-13), 5.89 (1H, s, H-5), 5.61 (1H, d, J = 2.1 Hz, H-13), 1.70 (3H, s, H-15), 1.02 (3H, d, J = 6.0 Hz, H-14); ^{13}C nmr ($CDCl_3$) δ 172.3 (s, CO), 141.2 (s, C-11), 133.9 (s, C-4), 129.9 (t, C-13), 121.6 (d, C-5), two singlets at 137.3 and 127.0 due to C-6 and C-7 (not assigned), 42.4 (d, C-1), 34.3 (d, C-10), 31.5, 31.1, 30.9, and 27.6 (4t, C-2, C-3, C-8, and C-9 not assigned), 23.9 (q, C-15), 20.3 (q, C-14) (5); eims *m/z* (rel. int.) $[M]^+$ 232 (53). *Anal.* calcd for $C_{15}H_{20}O_2$: C 77.55, H 8.68; found C 77.77, H 8.49.

OXIDATION OF ARTEMISINIC ACID [**3**] TO *EPI*-DEOXYARTEANNUIN B [**1**].—Artemisinic acid [**3**] (500 mg) was dissolved in CH_2Cl_2 (4 ml) and refluxed for 1 h with pyridinium chlorochromate (506 mg). Filtration and evaporation of the filtrate left 233 mg of residue that was flash chromatographed (2) using Me_2CO-C_6H_6 (1:99) as solvent to give 113 mg (23%) of pure **1** as colorless prisms from hexane, mp 81–82°, $[\alpha]_D$ +145° (c = 0.1, MeOH); eims *m/z* $[M]^+$ 232 (75%); ir, ^{13}C-nmr, and ^{1}H-nmr spectra indistinguishable from those of authentic *epi*-deoxyarteannuin B.

CONVERSION OF *EPI*-DEOXYARTEANNUIN B [**1**] TO 6,7-DEHYDROARTEMISINIC ACID [**2**].—*Epi*-deoxyarteannuin B [**1**] (40 mg) was dissolved in 0.5 ml of glacial HOAc, and the solution was heated at 100° for 1 h. The solvent was azeotroped by evaporation with a cyclohexane/C_6H_6 mixture, and the residue was flash chromatographed (2) on Si gel using Et_2O-hexane (1:4) as solvent to

give 30 mg (75%) of **2** as colorless prisms from Et_2O-hexane, indistinguishable from the natural material (same mp and mmp; superimposable ir, ms, and nmr spectra).

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