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A SIMPLE CONVERSION OF ARTEMISINIC ACID INTO ARTEMISININ

RONALD J. ROTH*

Department of Chemistry, George Mason University, Fairfax, Virginia 22030

and NANCY ACTON*

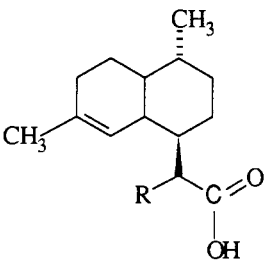
Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100

ABSTRACT.—Artemisinic acid [**1**] has been converted into artemisinin [**3**] in 2 steps via reduction of the exocyclic methylene group and photooxidation of the resulting dihydroartemisinic acid [**2**].

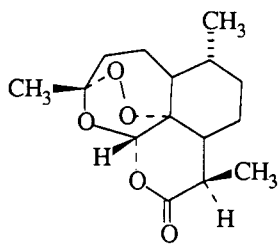
The photooxidation of artemisinic (arteannuic) acid [**1**], has been studied by several groups (1–3) in a search for a route from this relatively abundant constituent of *Artemisia annua* to the antimalarial drug artemisinin [**3**] (qinghaosu) which is present in the same plant in very low concentration (4). This photooxidation of **1** resulted in isolation of arteannuin B [**4**] and epideoxyarteannuin B [**5**], also *A. annua* constituents, but the hydroperoxide **6** that is expected to be the initial photooxidation product was not found. Our recent discovery that low temperature photooxidation of **1** allows isolation of this hydroperoxide (5) led us to investigate the same reaction with dihydroartemisinic acid, **2**.

Indeed photooxidation of **2** (6) (epimer mixture, −78°, CH_2Cl_2, methylene blue) rapidly destroyed the starting material with the major epimer disappearing faster than the minor. Allowing the crude photolysis mixture to stand at room temperature resulted in a remarkable transformation into artemisinin, **3**. Hplc analysis using electrochemical detection (eclc) (7) immediately after completion of the photooxidation revealed no artemisinin. That this compound is formed only on standing in air subsequent to the photooxidation was demonstrated by a 0% yield obtained in an argon-purged solution. The extent of conversion is solvent dependent, and in some solvents the rate is acid-catalyzed (see Experimental). Use of petroleum ether afforded the cleanest product as well as the highest yield of **3**. Thus, oxygen was passed through a −78° CH_2Cl_2 solution of **2** containing methylene blue while irradiating with a street lamp. After completion of the photooxidation, the CH_2Cl_2 was replaced with petroleum ether, and the mixture left at room temperature for 4 days. Removal of solvent followed by triturating with petroleum ether afforded crystalline artemisinin which was recrystallized from cyclohexane to give material identical in all respects (mp, specific rotation, ir, and nmr) with artemisinin isolated directly from *A. annua* (8). Flash chromatography of the mother liquors gave additional **3** for a total isolated yield of 17%.

Carrying out the photooxidation at 0°



1 R=CH_2
2 R=Me



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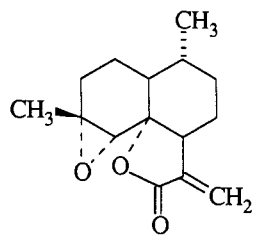

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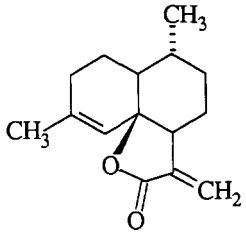

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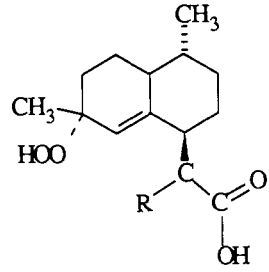


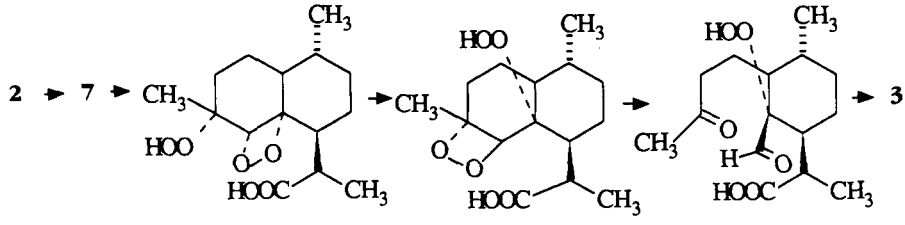
6 R=$=CH_2$
7 R=Me

or at room temperature also leads to artemisinin formation, but the yield is about half of that obtained at −78°. Low temperature Si gel chromatography of the −78° photooxidation mixture afforded a low yield of impure material assumed to be **7** because of the similarity of its nmr spectra with those of **6** (5). Although **7** is a plausible intermediate in the conversion of **2** into **3** via dioxetane formation followed by a rearrangement analogous to that reported by Jung *et al.* (6) for the synthesis of deoxyartemisinin (Scheme 1), allowing chromatographed **7** to stand in air (petroleum ether) resulted in formation of only a trace (ca. 1%) of **3**. Apparently **3** is produced by air oxidation of some other as yet unidentified photooxidation product of **1**.

Because there is far more **1** than **3** in *A. annua* [8–10 times more (6)] the conversion of **1** into **3** has received considerable attention. Indeed this transformation is part of a total synthesis of **3** reported in 1986 (9). The synthetic conversion was carried out in approximately 7% yield and involved 8 steps. Jung *et al.* (6) converted **1** into desoxyartemisinin in 4 steps but were unable to make artemisinin. Artemisinic acid has been isolated from *A. annua* by simple aqueous Na_2CO_3 extraction and has been purified without recourse to cc (10). Thus, even at 20% conversion, the artemisinic acid content of *A. annua* is a valuable resource which in two simple steps can more than double artemisinin production. Moreover, **3** can be obtained from the plant by this route with no cc required. This conversion also provides a short route to deuterium-labelled **3** via reduction of **1** with $NaBD_4$ (see Experimental). The facility of this photooxidation suggests that **1** precedes **3** in the plant biosynthesis. We are now attempting to isolate additional photooxidation product(s), to maximize the yield of **3**, and to incorporate radioactive labels into **3**.

EXPERIMENTAL

ARTEMISININ [**3**].—O_2 was passed through a −78° solution of **2** (1.0 g, 4.2 mmol, 5:1 epimer mixture) and methylene blue (6 mg) in 80 ml of CH_2Cl_2 while irradiating with a Westinghouse Ceramalux high intensity C400S51 electric discharge street lamp. After 90 min, hplc (11) showed almost no residual starting material (retention time 14.2 min, minor; 16.4 min, major epimer). Eclc (7) showed no artemisinin. Solvent was evaporated at room temperature, and the residue was taken up in Et_2O and filtered to remove most of the dye. Solvent was again removed, and the residue mixed with 150 ml of petroleum



SCHEME 1

ether. After standing at room temperature for 4 days, eclc indicated a 21% yield of artemisinin. The petroleum ether solution was decanted from insoluble material and the solvent evaporated. After redissolving in Et_2O, the solution was washed with 5% aqueous Na_2CO_3 (2 × 25 ml), H_2O (1 × 25 ml), and brine (1 × 25 ml). After drying ($MgSO_4$), solvent was removed, and the residue mixed with 10 ml of petroleum ether. Artemisinin (170 mg) crystallized from solution. Recrystallization from cyclohexane afforded 142 mg of **3**, mp 150–151° (uncorrected), $[\alpha]^{32}D$ +66° (*c* = 1.0, $CHCl_3$) [lit. (12) mp 156–157°, $[\alpha]^{17}D$ +66.3° (*c* = 1.64, $CHCl_3$)]. Flash chromatography of the mother liquors and the petroleum-ether-insoluble fraction [Si gel, Et_2O-petroleum ether (1:1)] followed by recrystallization as above afforded an additional 56 mg of **3** for a total yield of 17%.

The following effect of solvent on yield was determined by quantitative eclc (7): MeCN, EtOH, or Et_2O <1% after 3 days; CH_2Cl_2 13% after 18 h. Addition of a catalytic amount of CF_3CO_2H changed the yields as follows: MeCN 11% after 3 days; Et_2O ca. 2% after 18 h; petroleum ether 15% after 1 h, 24% after 18 h; CH_2Cl_2 11% after 18 h.

To demonstrate the requirement for O_2 in the conversion of the photooxidation mixture into **3**, two identical aliquots of photolysate in CH_2Cl_2 (−78°) were transferred to tubes. One was purged with argon, the other with O_2. The sealed tubes were allowed to warm to room temperature. After 5 days, eclc showed an 11% yield of **3** in the O_2-purged sample and 0% in the argon-purged sample.

ISOLATION OF HYDROPEROXIDE **6**.—Crude photolysate obtained from 500 mg of **2** was flash chromatographed using Et_2O as eluent on a column containing 150 ml of Si gel and cooled by circulating ice-H_2O. The chromatography was monitored by tlc [Si gel, Et_2O-cyclohexane (1:1)]. Fractions which exhibited a single tlc spot with R_f 0.2 were combined to afford 80 mg of a colorless gum: ir max ca. 3380–3020 (OH, COOH), 1707 (C=O), 1655 (C=C) cm^{-1}; ^1H nmr ($CDCl_3$, 90 MHz) δ 9.00 (2H, br s, OH and COOH), 5.24 (1H, br s, C=C*H*), 2.73 (1H, apparent p, *J* = 7 Hz, *CH*CO_2H), 1.30 (3H, s, CH_3COOH) superimposed on δ 1.26 (3H, d, $CH_3$$CHCO_2H$), 0.95 (3H, br d, *J* = 1.6 Hz, remaining Me); ^{13}C nmr ($CDCl_3$, 25 MHz) δ 15.6, 20.0, 22.7, 24.5, 28.9, 32.7, 35.5, 38.7, 41.2, 45.1, 47.2, 80.8, 120.3, 146.3, 182.2.

ARTEMISININ-*d*.—Reduction of **1** with $NaBD_4$ and $NiCl_2 \cdot 6H_2O$ as described for the protium analogue (6) afforded a quantitative yield of **2**-d_x. Gc-eims (70 eV) of the major gc peak showed small parent ions at *m/z* 237 and 238 in ca. 1:1 ratio. The ^1H nmr of this material showed a diminution and broadening in the area of the doublet seen at δ 2.62 for **2**. This material was photooxidized, then let stand in petroleum ether. After workup as above, recrystallization from Et_2O/petroleum ether, then from cyclohexane, afforded a 10% isolated yield of artemisinin-d_1, mp 145–146.5°, $[\alpha]^{24}D$ +66° (*c* = 1.0, $CHCl_3$). The ^1H nmr (220 MHz, $CDCl_3$) of this deuteriated material is identical with that of **3** (13) except for loss of fine structure (but not intensity) in the multiplet at δ 3.40 associated with the (exchangeable) proton α to the carbonyl and except for replacement of the sharp doublet at δ 1.22 associated with the lactone Me by a multiplet with intensity corresponding to ca. 2 protons. In the noise-decoupled ^{13}C nmr, the signal at 12.5 ppm associated with the Me in the lactone ring of **3** (13) has been replaced by a triplet of low intensity. Except for small differences in intensities, all other signals are the same as those in **3**.

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