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# ON THE POSSIBLE ROLE OF QINGHAO ACID IN THE BIOSYNTHESIS OF ARTEMISININ

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**Key Word Index**—*Artemisia annua*; Compositae; artemisinin; qinghao acid; arteannuin B; photo-oxygenation; HPLC; biosynthesis; rearrangement.

**Abstract**—Artemisinin (qinghaosu), a *seco*-sesquiterpene peroxide, is the clinically established antimalarial principle isolated from the leaves of the Chinese medicinal herb, *Artemisia annua*. Recent studies have suggested that arteannuin B, another metabolite of this plant, could serve as a precursor for artemisinin. In the present study, qinghao acid, the major sesquiterpene constituent of *A. annua*, was converted to arteannuin B by singlet oxygen ($^1O_2$) generated by sensitized photo-oxygenation. The formation of this compound was monitored by high-pressure liquid chromatographic analysis, and the identity of the isolated material was established by direct comparison. Since $^1O_2$ is known to play a role in biogenetic reactions, it appears that qinghao acid can serve as a biogenetic precursor for artemisinin.

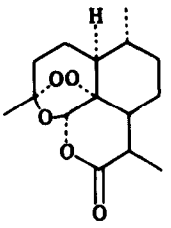
## INTRODUCTION

Artemisinin (qinghaosu) (**1**), isolated from *Artemisia annua*, is the active constituent of the traditional Chinese herbal medicine qinghao. Currently, there is intense interest in this clinically proven plasmocidal agent in view of its potency in cases of malaria that were resistant to traditional chemotherapeutic agents [1].

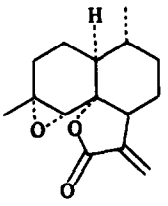
Recently, it was reported [2] that cell-free extracts of the leaves of *A. annua* converted arteannuin B (**2**), another metabolite of this plant, to artemisinin (**1**). However, a biosynthetic correlation with qinghao acid (**3**), a major sesquiterpene constituent of this plant, was never established. This paper describes the conversion of qinghao acid (**3**) to arteannuin B (**2**) by singlet oxygen ($^1O_2$) generated by dye-sensitized photo-oxygenation. The mechanism of this conversion and its biosynthetic implications in relationship to artemisinin (**1**) are discussed.

## RESULTS AND DISCUSSION

Qinghao acid (**3**), isolated from the leaves of a locally grown sample of *A. annua*, was subjected to methylene blue sensitized photo-oxygenation. The complex mixture* of products showed a spot corresponding to arteannuin B (**2**), $R_f$ 0.20, when analysed on silica gel G thin-layer chromatographic plates (see Experimental). The spot showed the same distinct turquoise colour as that of an authentic sample of arteannuin B (**2**) when sprayed with anisaldehyde spray reagent [3]. In addition, HPLC analysis on a microporasil column produced a peak, $R_t$ 3.73 min, identical to that of arteannuin B (**2**). Repeated flash chromatography [4] provided pure crystalline arteannuin B (**2**) in 12% yield, indistinguishable from an authentic sample.



**1**



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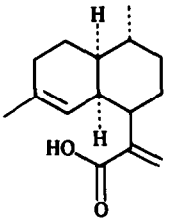

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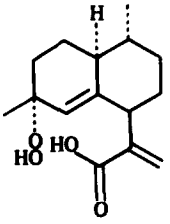
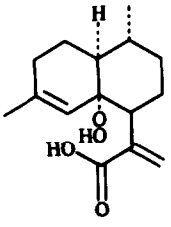


**4**



**5**

*A slightly impure, greenish spotting sample of an alcoholic solution of **3** was found to decompose to the same mixture spontaneously upon standing for several days.

The facile *in vitro* formation of arteannuin B (2) by the action of $^1O_2$ on qinghao acid (3) strongly suggests that similar conversion could take place *in vivo*; namely, in the leaves of *A. annua*. This notion is supported by what is known about the role of $^1O_2$ in biological oxidations [5], and specifically by the numerous examples of biogenetic-type syntheses of sesquiterpenes via hydroperoxides generated by sensitized photo-oxygenation [6]. This conclusion, therefore, would suggest that qinghao acid (3) is a possible precursor for the biosynthesis of artemisinin (1).

The formation of arteannuin B (2) from qinghao acid (3) by the action of $^1O_2$ cannot be readily rationalized on the basis of what is known about its site of attack; namely, at the olefinic double bond. Thus, to explain the formation of 2, an intermediate like 4 must have been formed first, then rearranged to 5, as is often reported [7] for allylic hydroperoxides. Epoxidation and deoxygenation of 5 followed by lactonization can then yield arteannuin B (2).

## EXPERIMENTAL

Mps: uncorr.; IR: KBr; $^1$H NMR: 100 MHz, $CDCl_3$, TMS as internal standard; $^{13}$C NMR: 25.0 MHz, $CDCl_3$, TMS as internal standard. TLC was performed on silica gel plates using *n*-hexane–$Et_2O$ (1:1) as solvent and visualized under short wavelength UV light or by spraying with anisaldehyde spray reagent [3]. The leaves of *A. annua* L. were obtained from plants grown at the garden of the Department of Pharmacognosy, College of Pharmacy, King Saud University. They were harvested on 15 December 1985 during the early flowering stage. At that time they contained 0.075% artemisinin (1) (isolated yield) [8] and 0.2% of arteannuin B as determined by HPLC analysis of a $CHCl_3$ soln of the plant material on a porasil column using 0.2% $CH_2Cl_2$ in *n*-hexane–$Et_2O$ (4:1) as the solvent system. The same sample of plant material was found to contain 0.3% of qinghao acid as determined by analysing an acetonitrile extract on a $C_{18}$ HPLC column using acetonitrile–0.5% HOAc in $H_2O$ (7:3) as the solvent system. Authentic samples of arteannuin B (2) and qinghao acid (3) were isolated from the plant material by a literature procedure [9].

*Photo-oxygenation of qinghao acid* (3). Qinghao acid (3, 1 g) was dissolved in 17 ml dry EtOH containing 1.5 mg methylene blue. The soln was subjected to 650 W incandescent light while a stream of $O_2$ was bubbling gently through it, and its temp. was maintained at 22° by cooling. The set-up used has been previously described [6]. After 4 hr, TLC showed the disappearance of 3 ($R_f$ 0.4) and the appearance of several spots, one of which corresponded to arteannuin B [same $R_f$ value (0.2), same turquoise colour with anisaldehyde spray reagent [3]]. Work-up as previously described [6] gave an oil (1.2 g) whose HPLC revealed the presence of 12% arteannuin B (2). Purification by chromatography on a chromatotron silica gel disc using *n*-hexane as solvent followed by flash chromatography [4] on silica gel using $Et_2O$–*n*-hexane (1:2) provided 71 mg colourless prisms of 2, mp 151–152°, $[\alpha]_D^{20}$ −7.3° (*c* 0.1; $CHCl_3$), lit. [10]: 152° and −6°, respectively. The identity was established further by comparison with an authentic sample of arteannuin B (same mmp and superimposable MS, IR and NMR spectra).

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