Journal of Natural Products
Vol. 47, No. 4, pp. 715-717, Jul-Aug 1984

ISOLATION OF ARTEMISININ (QINGHAOSU) FROM *ARTEMISIA ANNUA* GROWING IN THE UNITED STATES

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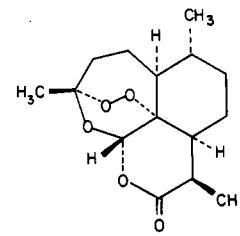
The herb, *Artemisia annua* L., a member of the Compositae family, has been used for many centuries in Chinese folk medicine. Ge Hong (1) in the year 340 and Li Shizhen (2) in 1596 recommended the taking of an aqueous infusion of the plant to alleviate the chills and fever of malaria. In 1967, a search was initiated in China for new antimalarial drugs that could be obtained from traditional remedies. Early studies, using extractions of *A. annua* with hot H_2O or EtOH, failed to confirm the curative properties of the plant. Later, however, extraction with Et_2O yielded a neutral fraction that possessed good antimalarial activity in mice infected with *Plasmodium berghei* and in monkeys infected with *Plasmodium cynomolgi* (1,3). In 1972, the active crystalline constituent was isolated from the aerial portions of the plant in 0.01-0.5% yield (4) by a procedure as yet unpublished. In 1979, its structure (**1**) was reported (5), and the compound was given various names by Chinese investigators, "artemisinine" (1), "qinghaosu" (i.e., active principle of qinghao) (3), and "arteannuin" (4). This sesquiterpene lactone has a peroxide function, destruction of which eliminates the antimalarial properties of the compound. Artemisinin[1] has been used to cure more than 2000 malaria patients infected with *Plasmodium vivax* and *Plasmodium falciparum* (3,6) and is reported to be efficacious against chloroquine-resistant *P. falciparum* as well as cerebral malaria (3,7). Many derivatives of **1** have been prepared, some of which surpass the parent in antimalarial potency (1, 8-11). The synthesis of artemisinin has been described recently (12).



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Some confusion exists as to the correct Chinese name for *A. annua*. Most of the current Chinese literature refers to the plant as *qinghao* (green herb); however, Read and Ju-ch'iang (13) called *A. annua, huang hua hao* (yellow flower herb), whereas *Artemisia apiacea* is called *chin hao*. Zhang (14), in 1981, expressed his agreement with the latter names and stated that it is misleading to call *A. annua, "qinghao"*. Still another source (15) refers to *Artemisia dracunculus* as *ch'ing hao*.

Because artemisinin has a structure totally different from existing antimalarials, we were prompted to ascertain to what extent the *A. annua* found in the United States contains this constituent. Thus, the plant, which grows nearby as a weed, was collected and air-dried. Several low boiling solvents (e.g., CH_2Cl_2, $CHCl_3$, Et_2O, Me_2CO) extracted **1** readily; however, petroleum ether (30-60°) was most selective and, therefore, considered to be the solvent of choice.

[1]Artemisinin (registry number 63968-64-9), rather than artemisinine, is the name preferred for **1** by *Chemical Abstracts*.

Treatment of the petroleum ether extract with CH_3CN removed much of the accompanying waxes, and further fractionation of the extract on silica gel gave **1**. Whereas the dried leaves and/or flowers yielded 0.06% of artemisinin, the stems of the plant were found to be devoid of the compound.

Chinese workers reported (4) that a study of 30 other *Artemisia* species (not identified) failed to uncover any with antimalarial properties. We examined ten species that grow in the United States by the extraction procedure outlined above and similarly did not find any containing **1**.

In view of the earlier reports of failure to detect antimalarial activity in EtOH extracts of *A. annua*, we examined the stability of **1** boiled in this solvent for 48 h. It is estimated by tlc that ca. 20% of the sample was destroyed by such treatment, as indicated by the appearance of a new spot at the origin. In addition, there was a broadening of the carbonyl peak at 1745 cm^{-1}. Treatment of **1** with *iso*-PrOH at reflux temperature for 48 h, however, led to the recovery of unchanged starting material.

Artemisinin was assayed in an *in vitro* system for antimalarial activity against chloroquine-susceptible and chloroquine-resistant strains of *P. falciparum* and showed potent inhibitory activity comparable to the quinolinemethanol, mefloquine. In an antibacterial screen, artemisinin displayed no inhibitory activity towards either Gram-positive or Gram-negative organisms.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Hoover-Thomas melting point apparatus and are uncorrected. Ir spectra were run as KBr disks on a Perkin-Elmer Model 283 spectrophotometer. The pmr and cmr spectra were run in $CDCl_3$ in a JEOL FX90Q spectrometer. Optical rotation was determined on a Perkin-Elmer Model 241MC polarimeter. Mass spectra were obtained on a Finnigan Model 3100D GC/MS operating in the CI mode (CH_4) using the solid probe.

PLANT MATERIAL.—The above-ground portions of *A. annua* used in this study were obtained in Virginia, Maryland, and the District of Columbia during the months of August and September 1983, and were identified by Prof. Ted Bradley, George Mason University, Fairfax, Virginia. Additional material was donated by Ms. Holly Shimizu of the National Herb Garden, U.S. National Arboretum, Washington, DC. The leaves and stems were air-dried and extracted separately. Samples of *Artemisia* species, namely, *A. pontica*, *A. abrotanum*, *A. pychnocephala*, *A. versicolor*, *A. schmidtiana*, *A. absinthium*, and *A. ludoviciana* were also provided by the U.S. National Arboretum. *A. tridentata* var. *tridentata*, *A. tridentata* var. *vaseyana*, and *A. arbuscula* were supplied by Mr. Robert Fuller, Bureau of Indian Affairs, Fort Duchesne, Utah. Samples of *A. vulgaris* were obtained in Maryland in September 1983, and *A. dracunculus* was purchased locally.

EXTRACTION AND FRACTIONATION.—Air-dried leaves of *A. annua* (200 g) collected in early August 1983, were extracted with boiling petroleum ether (bp 30-60°) for 48 h. Removal of the solvent *in vacuo* gave a dark brown syrup that was dissolved in 20 ml of $CHCl_3$ and to this solution was added 180 ml of CH_3CN. The insoluble material was removed, and the filtrate was evaporated under reduced pressure to give 4.5 g of gummy residue.

CHROMATOGRAPHIC SEPARATION.—The residue was chromatographed on 200 g of 70-230 mesh silica gel[2] using 7.5% EtOAc in $CHCl_3$ as the eluant. Fractionation was begun after passage of 200 ml of eluant and the aliquots were monitored by tlc (silica gel plates;[3] 7.5% EtOAc in $CHCl_3$; detection of **1** by I_2 vapor; Rf=0.66). Artemisinin appeared after collection of about 300 ml of eluant and was obtained (0.12 g, 0.06% yield) as fine white crystals, mp 153-154° [lit. (5) mp 156-157°], after recrystallization from cyclohexane. The identity of **1** with an authentic sample[4] of artemisinin was confirmed by comparative mp,[5] superimposable ir spectrum.[6] The pmr and cmr spectra, and $[\alpha]_D$ were identical to those reported (5).[7] Mass spectrum *m/z* 283 (M+1); calcd ($C_{15}H_{22}O_5$) 282.

A mixture of air-dried leaves and flowers of *A.*

[2]E. Merck, Darmstadt, F.R. Germany.

[3]Analtech, Newark, DE 19711.

[4]Obtained through the courtesy of Dr. W.H. Wernsdorfer, WHO, Geneva, Switzerland.

[5]Authentic sample melted at 151-153°.

[6]Reported by Liu *et al.* (5), lactone C=O at 1745 cm^{-1} (nujol); by the China Cooperative Research Group (11), C=O at 1735 cm^{-1} ($CHCl_3$); we found in the authentic sample and our product C=O at 1738 cm^{-1}.

[7]Found $[\alpha]^{20}D$ +69° (c=0.5, $CHCl_3$); Lit. (3) $[\alpha]^{17}D$ +66.3° (c=1.64, $CHCl_3$).

annua collected in early September 1983, when treated in the above-described manner, also yielded 0.06% of **1**.

The intrinsic antimalarial activity of artemisinin was quantitatively assessed *in vitro* using the semiautomated microdilution technique essentially that of Desjardins *et al.* (16). Based on the calculated 50% inhibitory concentration (IC_{50}), compound **1** was found to be equally effective (mean IC_{50}<3.4 ng/ml) against the chloroquine-susceptible (Camp) and chloroquine-resistant (Smith) isolates of *P. falciparum*. Note that mefloquine exhibits a mean IC_{50} of 3.5 ng/ml in the former strain and 2.5 ng/ml in the latter, whereas chloroquine, an IC_{50} of 7.6 ng/ml against the Camp and an IC_{50} of 60 ng/ml against Smith strains (17).

The antibacterial activity of **1** against clinically significant organisms was evaluated using microtiter plates with a Mueller-Hinton broth microdilution procedure. Included in the screen were the following Gram-positive organisms [number of strains]: *Staphylococcus aureus* [5], *Streptococcus faecalis* [5]; and the following Gram-negative organisms: *Klebsiella-Enterobacter* [7], *Shigella dysenteriae* [4], *Escherichia coli* [5], *Serratia marcescens* [8], and *Proteus* spp. [8]. The minimum inhibitory concentration (MIC) in each case was >32 μg/ml. In the assay of *Neisseria meningitidis* [5], another Gram-negative genus, the MIC was >1 μg/ml. *N. gonorrhoeae* [25], tested with a supplemented GC agar dilution procedure (18), also had an MIC >1 μg/ml. Artemisinin appeared to encourage the growth of *N. gonorrhoeae*.

ACKNOWLEDGMENTS

We are grateful to Prof. Ted Bradley of the Department of Biology, George Mason University, Fairfax, VA, for guiding us to our first *A. annua* growing in the wild. Also, we are pleased to acknowledge the excellent cooperation of Ms. Holly Shimizu who provided additional quantities of the plant and specimens of other *Artemisia* species, and Mr. Robert Fuller, for samples of sagebrush growing in Utah. We sincerely thank our numerous enthusiastic co-workers for their aid in collecting plant material from the field and especially Irvin Rollman, for assistance in processing it. Finally, we appreciate the suggestions and interest of Dr. Craig J. Canfield.

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Received 11 November 1983