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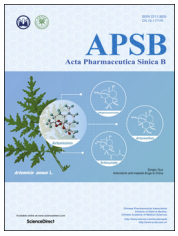

FEATURE

Artemisinin anti-malarial drugs in China

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Artemisinin;
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Abstract Discovered by Youyou Tu, one of the 2015 Nobel Prize winners in Physiology or Medicine, together with many other Chinese scientists, artemisinin, artemether and artesunate, as well as other artemisinins, have brought the global anti-malarial treatment to a new era, saving millions of lives all around the world for the past 40 years. The discoveries of artemisinins were carried out beginning from the 1970s, a special period in China, by hundreds of scientists all together under the "whole nation" system. This article focusing on medicinal chemistry research, briefly introduced the discovery and invention course of the scientists according to the published papers, and highlighted their academic contribution and achievements.

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1. Background of artemisinin research

1.1. Discovery of new anti-malarials in a whole nation system

During the Vietnam–US War in the early 1960s, Vietnamese soldiers suffered from serious malaria because a mutated form of *Plasmodium falciparum* wreaked havoc in Vietnam. Under the request from North Vietnamese government to provide effective drugs against multidrug-resistant malaria, China started, on May 23, 1967, a project to search for new antimalaria drugs; the project, named "Project 523", was managed under the "whole nation" system, and it involved sixty research organizations and more than 500 scientists[1,2]. All activities of the project were directed by the entity known as "Project 523".

1.2. The program for new drugs or lead compounds from traditional Chinese medicine (TCM) and folk medicine

One of the various programs in the "Project 523" was a team for the "study and survey on effective folk medicine and therapy against malaria". Making every endeavor, the team obtained several hits, including a sesquiterpene, yingzhaosu A, from *Artabotrys hexapetalus*, a metal-containing principle in the plant *Polyalthiane moralis*, analogs of β-dichroine and components from *Artemisia*[3].

1.3. The discovery of artemisia and artemisinin

In 1969, the Institute of Chinese Materia Medica (ICMM), China Academy of Traditional Chinese Medicine, joined the "Group of TCM" of Project 523. Youyou Tu of ICMM was the group leader. She and her ICMM colleague Yagang Yu and Guoming Gu of Academy of Military Medical Sciences (AMMS), collected and screened more than 100 simple and compound recipes from well-recorded folk medicines and TCM, where they found that *Artemisia* appeared in high frequency for recorded efficacy against malaria (anti-malarial action of *Artemisia* was recorded in many ancient Chinese herb books in Dynasties Tang, Song, Yuan and Ming). Through systematic bioassay-guided screening by Yu and Gu, an alcoholic extract of *Artemisia* was discovered to exert inhibitory activity against *Plasmodium falciparum* by up to 60%–80%[1]. The extent of the inhibition was unfortunately highly variable; however, the results from Yu and Gu provided valuable references for further investigations.

Critical progress was subsequently made by Youyou Tu, who was inspired by the detailed methods of *Artemisia* usage described in ancient Ge Hong's book "*Zhou Hou Bei Ji Fang*". The book reads: for the treatment of malaria "a handful of *Artemisia* soaked in two liter of water. Take the pressed juice." She deduced that the disuse of decoction (by boiling) may imply thermo-instability of active principles, which nonetheless may be lipophilic. Therefore, she switched from ethanol to ether as the extraction solvent. After removing the acidic principles, white solids were isolated from the neutral ethereal extraction, which exerted 100% inhibition against mouse *P. falciparum*. The white solids were later identified to be artemisinin (**1**) (Fig. 1). The discovery of artemisinin from the ethereal extraction certainly played a critical role in opening new therapeutic means and saving millions of lives from malaria illness. It also justifies Youyou Tu's sharing of the 2015 Nobel Prize for Physiology or Medicine.

In addition to artemisinin, several other sesquiterpenes were identified from the ethereal extraction, including arteannuic acid (**2**), arteannuin A (**3**), arteannuin B (**4**), arteannuin C (**5**), and amorphane (**6**) (Fig. 1). However, these compounds all exerted weak or no anti-malaria activity[4].

2. Structural identification of artemisinin

2.1. Physicochemical properties

Artemisinin is white needle-like crystals with mp 151–153 °C. Elementary analysis and mass spectra showed the molecular formula of $C_{15}H_{22}O_5$. It is insoluble in water, but dissolves in acetone, ethanol, ether, petroleum ether and alkali solution. NaOH titration of artemisinin consumes one equivalent. Qualitative analyses give positive color reactions in the oxidation of $FeCl_2$ or NaI. It quantitatively reacts with triphenylphosphine to give one equivalent of triphenylphosphine oxide. These reactions indicated the existence of an oxidative group in its molecule.

2.2. Spectral behavior

The ultraviolet spectra (UV) of artemisinin showed the absence of an aromatic conjugate system. The infrared spectra (IR) indicated carbonyl peak of δ-lactone. ^{13}C NMR revealed the presence of fifteen carbon signals, the numbers of primary, secondary, tertiary and quaternary carbon being 3, 4, 5 and 3, respectively. For the quaternary carbons, one yielded a carbonyl signal, whereas the other two were at low field (79.5 and 105 ppm) , indicating linkage to an oxygen atom. Five doublet signals of tertiary carbon atoms appeared at the high field. ^1H NMR showed a singlet at 5.68 ppm, indicating the presence of an –O–CH–O– fragment. Based on the biogenesis of sesquiterpene, the 4 oxygen atoms were deduced as ketal, acetal and lactone moieties in the artemisinin scaffold. Yet the 5th oxygen was not defined.

At that time (1975), Dequan Yu, a member of the TCM group of Project 523 at the Institute of Materia Medica, CAMS, promulgated the chemical structure of another anti-malarial natural product, yingzhaosu A , which contains a peroxy linkage[5]. This information conferred an enormous enlightenment for solving the artemisinin structure. Based on the oxidation of artemisinin in qualitative and quantitative analyses, a peroxy moiety was designated in the structure, at three possible locations, as shown in **1**, **7** and **8** (Figs. 1 and 2).

The location of the peroxy linkage and the structure of artemisinin were authentically defined by the method of X-ray crystallography in the Institute of Biophysics, Chinese Academy of Scince (CAS). The absolute configuration was determined by optical rotatory dispersion (ORD)[6–8].

2.3. Chemical reactions of artemisinin—evidence in support of structure

Hydrogenation of artemisinin, under the catalyst of $Pd/CaCO_3$, gave rise to a product, coined reduced artemisinin, with the peroxy moiety being reduced into ether linkage. The structure of reduced artemisinin is identical to that of arteannuin C (**5**). The mechanism of the reduction is illustrated in Scheme 1.

Artemisinin reacts with $NaBH_4$ under low temperature to produce dihydroartemisinin (**9**, Scheme 2), in which the C10-carbonyl group is reduced into hydroxyl in the form of hemi-acetal. In the presence of Lewis acid with the treatment with $NaBH_4$ the C10-carbonyl group of artemisinin converts into methylene group (**10**, Scheme 2); artemisinin reacts with acetic-sulfuric acid to generate (**11**), where the