REVIEW ARTICLE

The Antiviral Activities of Artemisinin and Artesunate

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Traditional Chinese medicine commands a unique position among all traditional medicines because of its 5000 years of history. Our own interest in natural products from traditional Chinese medicine was triggered in the 1990s, by artemisinin-type sesquiterpene lactones from *Artemisia annua* L. As demonstrated in recent years, this class of compounds has activity against malaria, cancer cells, and schistosomiasis. Interestingly, the bioactivity of artemisinin and its semisynthetic derivative artesunate is even broader and includes the inhibition of certain viruses, such as human cytomegalovirus and other members of the *Herpesviridae* family (e.g., herpes simplex virus type 1 and Epstein-Barr virus), hepatitis B virus, hepatitis C virus, and bovine viral diarrhea virus. Analysis of the complete profile of the pharmacological activities and molecular modes of action of artemisinin and artesunate and their performance in clinical trials will further elucidate the full antimicrobial potential of these versatile pharmacological tools from nature.

Artemisinin is a natural product derived from the Chinese herb *Artemisia annua* (figure 1). During the Vietnam War, Ho Chi Minh asked Mao Zedong for help, because more North Vietnamese soldiers were dying from malaria than from armed conflicts. The Chinese government launched a program to find new antimalarial drugs. As a result, Tu Youyou, a Chinese scientist from the Chinese Academy of Traditional Chinese Medicine (Beijing, China), identified artemisinin as the active compound of *A. annua* in 1972 [1]. Its overwhelming antimalarial activity was demonstrated in numerous clinical studies by Chinese and Western scientists. Despite this success, the true potential of artemisinin was underestimated in the Western world for many years [2]. In the meantime, the World Health Organization officially recommends artemisinin and its derivatives, such as artesunate and artemether, for the treatment of malaria, particularly as a part of combination therapies with other antimalarial drugs.

Artemisinin, artesunate, and additional derivatives are the most promising candidate compounds to ease the worldwide malaria burden. The high safety and tolerability profile of these drugs adds to their attractiveness [3]. This group of compounds is also active against cancer cells and schistosomiasis [4–10]. The focus of the present review is the antiviral activity of artemisinin and artesunate.

Although some authors claim that the heme-mediated decomposition of the endoperoxide bridge and production of carbon-centered free radicals is necessary for antimalarial activity [11], other data indicate that the biological activity of artemisinin-like drugs does not correlate with their chemical reactivity [12]. Computer-assisted models for the calculation of quantitative structure-activity relationships have been developed to address these contradictory results [13–15].

Peak plasma concentrations of 391–588 μg/L have

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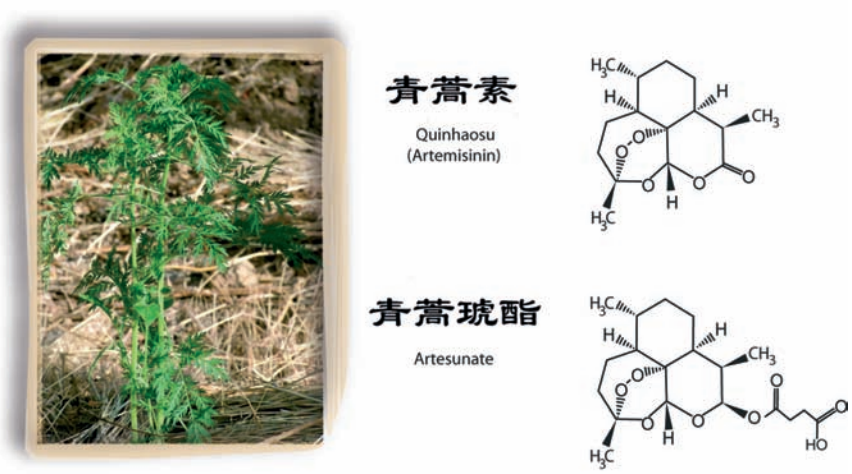

Figure 1. Cultivar of the Chinese herb *Artemisia annua* L. (qinhao or sweet wormwood) and its active principle artemisinin (qinhaosu). Artesunate represents a semisynthetic derivative of artemisinin that has improved water solubility.

been reported for orally administered artemisinin (500 mg, single dose) [16, 17], and peak plasma concentrations of 2640 μg/L and 2020 μg/L have been reported for intravenous artesunate (2 mg/kg) and for its active metabolite dihydroartemisinin, respectively [18, 19]. The oral administration of artesunate (100 mg) leads to a plasma-elimination half-life of 39–95 min for dihydroartemisinin [20–26].

The metabolism of artemisinin in human liver microsomes is primarily mediated by cytochrome P-450 monooxygenase enzyme (CYP) 2B6, with a secondary contribution by CYP3A4 in individuals with low CYP2B6 expression. The contribution of CYP2A6 to artemisinin metabolism is likely of minor importance [27]. There is a large body of evidence suggesting that artemisinin influences the CYP activity, which could result in drug-drug interactions [28]. An induction of activity by artemisinin was reported for CYP2A5, CYP2A6, CYP2B1, CYP2B6, CYP2B10, CYP2C19, and CYP3A4 [29–34]. In addition, artemisinin activates the constitutive androstane receptor and pregnane X receptor [33, 34], which explains the upregulation of CYP2B6 and CYP3A4. The data regarding CYP1A2 are contradictory [35–38], whereas artemisinin inhibits CYP2D6 [37]. Artemisinin leads to an autoinduction of drug metabolism, which reduces its own bioavailability [39].

In various clinical studies, artemisinin has been administered alone or in combination with other antimalarial drugs in dosages of up to 500 mg per day. As reviewed elsewhere, clinical trials of artesunate monotherapy used dosages of 1–8 mg/kg intravenously or 600–1200 mg per day orally for 5 days [40]. In combination therapies, 4–25 mg/kg intravenously or 200–800 mg per day orally for 3 days have been used [40].

Artemisinin derivatives are tolerated well by patients [41, 42]. Mild and reversible hematological and electrocardiographic abnormalities, such as neutropenia and first-degree heart block, have been observed infrequently. Neurotoxic effects have been repeatedly reported in experiments with mice, rats, and dogs, as reviewed elsewhere [43]. Affected areas in the brain stem are the reticular system with regard to autonomic control, the vestibular system, the auditory system (trapezoid nucleus), and the red nucleus, which is important for coordination [44–51]. A longer exposure time to a lower peak blood concentration of an artemisinin derivative is more neurotoxic than a shorter duration of exposure and a higher peak blood concentration [52]. These animal experiments gave rise to concerns about the safety of artemisinin and its derivatives in humans. A clinical safety review of 108 clinical studies that enrolled 9241 malaria patients provided ample evidence that artemisinins are safe and without serious adverse effects or significant severe toxicity, including neurotoxicity [41]. Ataxia, slurred speech, and hearing loss have been reported in few patients treated with artemisinin [53]. Although the artemisinin derivative artesunate seems to be without toxicity, van Hensbroek et al. [54] observed delayed coma recovery times in Gambian children with malaria who were treated with intramuscular artemether versus intravenous quinine. Because of these conflicting results, Stepniewska et al. [55] performed a meta-analysis of 7 studies involving 1919 patients with malaria. Applying a uniform coma recovery time definition, no significant difference in coma recovery time was found between patients treated with artemether and quinine. Additionally, no statistically significant difference was observed with regard to neurological sequelae. In a recent study by Dondorp et al. [56], patients with malaria who were treated with artesunate were compared with patients who were treated with quinine. The authors did not find significant differences in terms of neurotoxic symptoms (i.e., times to speak, eat, and sit) between treatment groups. Neurological sequelae did not occur after treatment. Interestingly, patients with ma-

laria who developed late onset hypoglycemia had a higher incidence of death than did patients treated with artesunate who did not have hypoglycemia. This may be an issue that deserves additional investigation.

ACTIVITY AGAINST HUMAN CYTOMEGALOVIRUS (HCMV)

Chinese scientists provided the first hint that artemisinin might have antiviral activity [57]. Indeed, artesunate inhibits the in vitro replication of HCMV (HCMV AD169 and other strains; table 1) and herpes simplex virus type 1 (HSV-1) [59]. With regard to artesunate's potential inhibition of HCMV, it was important to demonstrate that viruses with a variety of phenotypes (i.e., low-passage clinical isolates, drug-resistant mutants, laboratory strains, and recombinant virus clones) were all highly sensitive to artesunate. A possible mechanism was suggested by the finding that artesunate inhibited central regulatory processes of HCMV-infected cells (such as activation pathways dependent on NF-κB or Sp1), thus interfering with critical host-cell–type and metabolism requirements for HCMV replication.

HCMV is a major cause of disease in immunocompromised individuals, including patients with AIDS and transplant recipients, and it is a common cause of congenital infection leading to developmental abnormalities and hearing loss [62]. All currently available anticytomegaloviral drugs, including ganciclovir, foscarnet, and cidofovir, target the viral DNA polymerase. The use of these drugs is limited by toxicity, low oral bioavailability (with the exception of the oral prodrug valganciclovir), teratogenicity, and drug resistance. These limitations, along with the repeated and prolonged courses of therapy often required for the treatment of HCMV infection in transplant recipients, create an increasing need for new antiviral drugs, particularly for drugs that exhibit low levels of toxicity and activity against HCMV variants that are resistant to conventional drugs [59].

The replication of HCMV is tightly coregulated with cellular activation pathways mediated by the direct or indirect interaction with cellular DNA-binding factors, such as NF-κB and Sp1 [63, 64]. These factors provide major determinants of the virus–host cell interaction. For both NF-κB and Sp1, a reduction in HCMV-induced protein synthesis and a reduction in the DNA binding activity of NF-κB and Sp1 were observed with artesunate treatment [59]. The inhibitory activity towards NF-κB is not specific for artesunate alone; it has also been demonstrated for other sesquiterpene lactones [65, 66]. The efficiency of HCMV replication is closely connected with NF-κB and Sp1 activation pathways and other involved factors, such as the cellular signaling kinase phosphoinositol 3–kinase [67]. Phosphoinositol 3–kinase is required for the activation of NF-κB and Sp1 in infected fibroblasts. Interestingly, the phosphorylation of downstream effectors of phosphoinositol 3–kinase, such as the protein kinases Akt and p70S6K, is also inhibited by artesunate [59] (figure 2). Moreover, there are several examples that chemical compounds interfering with activation pathways of cellular transcription factors (e.g., the signal transduction pathway that includes mitogen-activated protein kinase p38) inhibit HCMV replication. It is noteworthy that the HCMV immediate-early promoter enhancer (in addition to other viral promoters) contains binding sites for both Sp1 and

Table 1. Sensitivity of herpesviruses to artesunate.

Sensitivity test	Herpesvirus type	Herpesvirus subfamily	Strain or isolate	Type of analysis	Inhibition at 15 μM, %	IC_{50}, μM	Reference
1	HCMV	β	AD169	In vitro[a]	…	3.9 ± 0.6	[58]
2	HCMV	β	AD169	In vitro[a]	81	5.8 ± 0.4	[59]
3	HCMV	β	Towne	In vitro[a]	>99	…	[59]
4	HCMV	β	Clinical isolates	In vitro[a]	82	…	[59]
5	HCMV	β	Clinical isolates	In vitro[a]	69	…	[59
6	HCMV	β	Ganciclover resistant mutant	In vitro[a]	…	6.9 ± 0.2	[59]
7	HCMV	β	Multidrug resistant mutant	Clinical trial[b]	…	…	[60]
8	RCMV	β	Maastricht	In vitro[a]	38	…	[58]
9	RCMV	β	Maastricht	In vivo model[c]	…	…	[58]
10	MCMV	β	Smith	In vitro[a]	15	…	M.M., unpublished data
11	HHV-6A	β	U1102	In vitro[d]	76	3.80 ± 1.06	M.M., unpublished data
12	HSV-1	α	Clinical isolate	In vitro[a]	83	…	[59]
13	EBV	γ	B95-8	In vitro[d]	63	7.21 ± 2.25	M.M., unpublished data

NOTE. EBV, Epstein-Barr virus; HCMV, human cytomegalovirus; HHV-6A, human herpesvirus 6A; HSV-1, herpes simplex virus 1; MCMV, murine cytomegalovirus; RCMV, rat cytomegalovirus.

[a] Virus replication analyzed in infected primary fibroblasts (green fluorescent protein–based reporter assays and plaque reduction assays) [61].

[b] Clinical trial with HCMV-infected patients after hematopoietic stem cell transplantation (quantitative PCR and antigenemia assays for viral load in blood specimens) [60].

[c] Virus replication analyzed in immunosuppressed rats (quantitative PCR and plaque reduction assays of salivary gland specimens) [58].

[d] Virus replication analyzed in infected immortalized lymphocytes (quantification of immunofluorescence stainings).

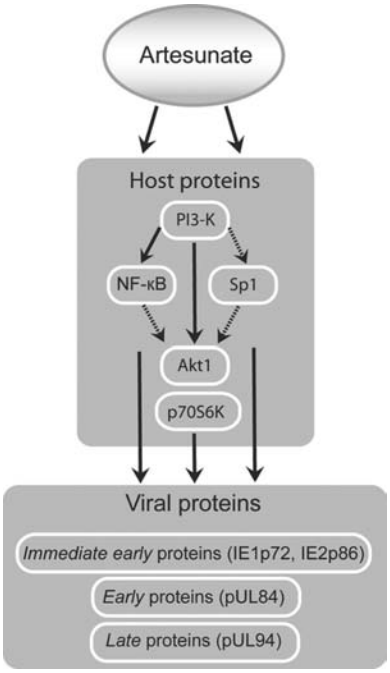

Figure 2. Hypothetical molecular mechanism of artesunate against human cytomegalovirus.

NF-κB and, therefore, is responsive to both factors [68, 69]. Reduction of IE2p86 expression critically limits viral replication, because IE2p86 is essential for the initiation of subsequent regulatory steps [70]. On the other hand, NF-κB is a major factor in cellular defense pathways (e.g., IFN type 1-induced antiviral effects) and may also have a negative impact on viral productivity and the course of infection [71]. Thus, the activation pathways that involve Sp1 and NF-κB are important factors in the initial onset of the viral replication cycle, as well as in later steps in virus-cell interaction, and are, therefore, crucial for the antiviral action of artesunate.

Because of this background, it was interesting to analyze whether artesunate was active against drug-resistant HCMV, as well. Indeed, ganciclovir-resistant mutants (i.e., the laboratory mutant AD169-GFP314, which carries the resistance-conferring mutation UL97 [M460I], or ganciclovir-resistant clinical isolates) were inhibited with similar efficacy as the drug-sensitive parental virus (AD169-GFP) [61]. It is obvious from the results of these experiments involving ganciclovir-resistant HCMV that the putative inhibitory mechanisms of artesunate must be different than the mechanism of conventional DNA polymerase–inhibiting drugs.

The anticytomegaloviral activity of artesunate is not restricted to HCMVs but also includes animal CMVs—in particular, rat CMV [58]. An important finding was that increased intracellular iron concentrations enhance artesunate's anticytomegaloviral activity. This iron-enhanced effectiveness was demonstrated by several observations, and the following are some promising features of the anticytomegaloviral activity of artesunate. First, treatment of CMV-infected fibroblasts with artesunate plus ferrous iron (Ferrosanol; Monheim) and/or soluble Transferrin resulted in enhanced suppression of viral replication. Because Ferrosanol is a clinically approved formulation, this drug could potentially be safely combined with artesunate in clinical practice. Second, the antiviral activity of artesunate is additive in combination with conventional drugs, such as ganciclovir, cidofovir, and foscarnet. A combination of drugs with different modes of action may delay the development of drug resistance. Third, the antiviral activity of artesunate against CMV was also demonstrated in vivo using the rat CMV model. Importantly, the first successful clinical use of artesunate for the treatment of HCMV in a patient who developed drug-resistant infection during preemptive antiviral therapy after stem cell transplantation has been described [60]. In this case, artesunate proved to be a highly effective and well-tolerated inhibitor of HCMV replication, which suggests the need for additional clinical evaluation of its role in the treatment of HCMV infection.

BROAD-SPECTRUM ACTIVITY AGAINST HERPES VIRUSES

The antiviral activity of artesunate is not restricted to distinct viral laboratory strains; artesunate is also effective against clinical isolates of HCMV and mutants with resistance against conventional antiviral drugs, such as ganciclovir and cidofovir (M. Leis and M.M., unpublished data). Novel data show that other herpesviruses from all subfamilies (α, β, and γ) are also sensitive to artesunate—namely, Epstein-Barr virus, herpes simplex virus 1, and human herpes virus 6A (table 1; M.M., unpublished data) [58, 59]. This finding suggests that artesunate has broad activity against herpesviruses. The herpesviruses that have been analyzed thus far have all demonstrated similar sensitivity to artesunate (IC_{50}, <10 μM). Some of the analyzed herpesviruses show different sensitivities to artesunate and artemisinin; artemisinin is inactive against human herpesvirus 6A [72] and has poor activity against HCMV [58]. This indicates that the semisynthetic drug artesunate has more antiherpesviral potency than does its natural parental drug, artemisinin.

ACTIVITY AGAINST HEPATITIS B VIRUS (HBV)

The family *Hepadnaviridae* includes a group of highly species-specific viruses that all have a virus-encoded DNA polymerase with reverse-transcriptase activity [73–75]. One member of this family, human HBV, is characterized by a high level of hepatotropism. This virus belongs to the genus *Orthohepadnavirus* and is not cytopathic itself, although it may cause acute fulminant hepatitis [76] or chronic liver disease, which may progress to cirrhosis and, eventually, hepatocellular carcinoma [77]. In spite of the availability of an effective and safe vaccine

against HBV, infection by this virus has remained a major worldwide health problem [78, 79]. Although several pharmacological strategies are currently being implemented to treat HBV-infected patients (i.e., the use of IFN and a nucleoside derivative, lamivudine), no effective antiviral therapy against HBV infection has yet been fully developed.

In a recent investigation [80], a panel of natural products derived from medicinal herbs used in traditional Chinese medicine has been assayed for anti-HBV activity. Among these products, artesunate displayed anti-HBV activity. HBV DNA release was inhibited at an IC_{50} of 0.5 μM. Host cell viability was reduced at a concentration 40-times greater (20 μM). Moreover, the treatment potential is enhanced by synergistic effects with lamivudine and by the absence of drug-induced toxicity in host cells. This is important in clinical practice because of frequent cases of infection by lamivudine-resistant HBV strains.

The concentration at which artesunate was active against HBV (>10 μM) was similar to that previously reported for its activity against HCMV [59]. Interestingly, these levels are close to the drug concentrations achieved in the plasma of patients in whom this drug is used for anti-malarial treatment (~7 μM) [81]. This result was similar to that reported elsewhere [82] for artesunate use in HepG2 2.2.15 cells.

ACTIVITY AGAINST HEPATITIS C VIRUS (HCV) AND RELATED VIRUSES

The family *Flaviviridae* includes 3 genera: *Pestivirus* (e.g., bovine viral diarrhea virus), *Flavivirus* (e.g., Japanese encephalitis virus), and *Hepacivirus* (e.g., HCV). Pathogens of the family *Flaviviridae* constitute a major cause of disease worldwide. Infection with HCV frequently causes chronic hepatitis, which may progress to cirrhosis and hepatocellular carcinoma [83]. The problem is aggravated by the absence of an efficient vaccine against HCV and because the standard treatment (pegylated IFN-α and the purine nucleoside analogue ribavirin [1β-*D*-ribofuranosyl-1,2,4-triazole-3-carboxamide]), in addition to having adverse effects, is not effective in approximately one-half of infected patients [83]. Therefore, the search for more effective therapies is crucial. Because all members of the *Flaviviridae* share similarities in virion structure, genome organization, and replication machinery, some viruses, in particular bovine viral diarrhea virus, have been used as in vitro models [84].

The pharmacological interest in artemisinin and its derivatives for the treatment of infections by these viruses is increased by the severe limitations of currently available antiviral therapy. Because the mechanisms of action of IFN-α [85, 86] and ribavirin [86, 87] against *Flaviviridae* viruses are probably different than the mechanisms of artemisinin [88], it was possible that a combination of these drugs would demonstrate additive effects; indeed, additive effects were observed by Romero et al. [89]. IFN binds to cell surface receptors and stimulates signal pathways that lead to the activation of cellular enzymes that repress viral replication [85], whereas ribavirin, in addition to its immunomodulatory properties, has direct antiviral activities that can be ascribed to several possible mechanisms. These mechanisms include the inhibition of the HCV RNA–dependent RNA polymerase NS5B and ribavirin's activity as an RNA mutagen, which enables it to impair viral replication [90]. Paeshuyse et al. [91] reported that the antimalarial drug artemisinin inhibited HCV replicon replication in a dose-dependent manner in 2 HCV subgenomic replicon constructs at concentrations that did not affect Huh 5–2 host cells. Hemin, an iron donor, inhibits HCV replicon replication by inhibiting the viral polymerase [92]. The combination treatment of artemisinin and hemin had a pronounced synergistic antiviral activity without affecting host cells.

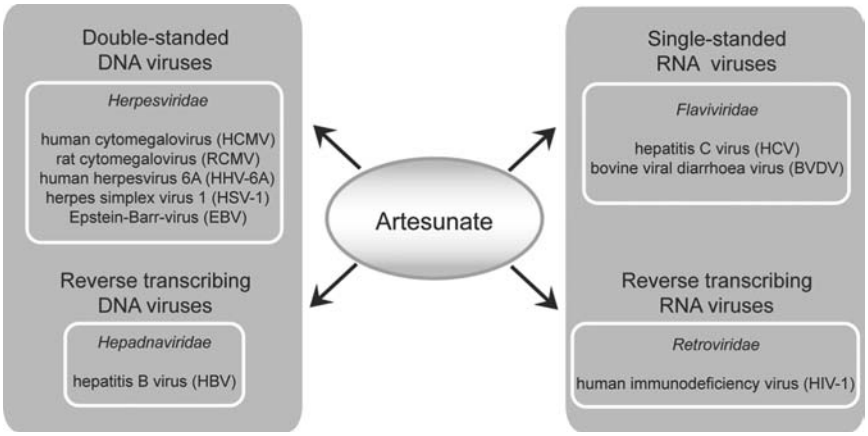

Figure 3. Spectrum of antiviral activity of artesunate

ACTIVITY AGAINST HIV-1

Artesunate has been observed to have activity against HIV-1, as reported elsewhere [59]. Partial inhibition was demonstrated for 2 strains of HIV-1—the CCR5-tropic (M-tropic) HIV-1 strain Ba-L (in PM1 cells) and the CXCR4-tropic (T-tropic) HIV-1 strain NL4–3 (in Jurkat cells). The replication of both HIV-1 strains was partially inhibited by 600 nM artesunate throughout the analyzed time period of 10 days. Birku et al. [93] investigated the effect of artemisinin on the rate of clearance of *Plasmodium falciparum* in patients with or without HIV coinfection. Interestingly, Birku et al. [93] observed that HIV-infected patients showed a delayed clearance of *P. falciparum,* which suggested that the health of the host's immune system affects the activity of antimalarial drugs. No anti-HIV activity of artemisinin was reported in this investigation.

CONCLUSIONS AND PERSPECTIVES

After being used in traditional Chinese medicine for 2 millennia, 1 of the "gems" of traditional Chinese medicine's treasure box has been rediscovered during recent years. Artemisinin is certainly one of the most promising natural products investigated in the past 2 decades. With regard to malaria, artemesinin has the potential to considerably contribute to a change in the desperate situation that the world is facing. Fortunately, the value of this compound is not limited to the treatment of malaria, and a wealth of studies have demonstrating the activity of artemisinin and its derivatives against cancer cells, schistosomiasis, and as reviewed here, various viral diseases (figure 3).

Ironically, in an age in which many scientists are searching for compounds with increased specificity to their molecular and cellular targets, awareness of artemisinin is increasing because of its multifunctionality. This class of compounds seems to have several targets that are important for different diseases. Conceptually, modern projects in molecular pharmacology aim to increase treatment efficacy and to decrease unwanted side effects by developing compounds that attack disease-related target molecules with high affinity. It is obvious that the natural evolution of pharmacologically active compounds in plants evolved in a different way. Natural products have evolved in plants as chemical weapons to protect against infections by bacteria, viruses, and other microorganisms. It is no surprise that multifunctional molecules might be more versatile and, therefore, more successful than monospecific molecules for protecting plants from environmental harm. In the case of artemisinin, it has been shown that it is active against various plant pathogenic fungi (i.e., *Gaeumannomyces graminis* var. *tritici, Rhizoctonia cerealis, Gerlachia nivalis,* and *Verticillium dahliae*) [94], which supports the role of artemisinin as a protective agent for the plant. This view of chemical evolution in plants may fertilize current scientific concepts.

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