Pesticide Biochemistry and Physiology 143 (2017) 252–257

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Recent advances in tenuazonic acid as a potential herbicide

Shiguo Chen, Sheng Qiang *

Weed Research Laboratory, Nanjing Agricultural University, Nanjing 210095, China

ARTICLE INFO

Article history:
Received 19 September 2016
Received in revised form 27 December 2016
Accepted 3 January 2017
Available online 4 January 2017

Keywords:
Mycotoxin
Tetramic acid
Mode of action
Bioherbicide

ABSTRACT

Tenuazonic acid (TeA), belonging to tetramic acids that are the largest family of natural products, is a mycotoxin produced by members of the genus *Alternaria* and other phytopathogenic fungi. TeA has many desirable bioactivities. In the past two decades, several studies have addressed its phytotoxic activity. Because it can cause brown leaf spot and kill seedlings of mono- and dicotyledonous plants, TeA is regarded as a potential herbicidal agent. TeA blocks electron transport beyond Q_A by interacting with D1 protein and is a *PSII* inhibitor. The chloroplast-derived oxidative burst is responsible for TeA-induced cell death and plant necrosis. Based on the model of molecular interaction between TeA and D1 protein, a series of its derivatives with stable herbicidal activity have been designed, evaluated and patented. Recently, some chemical synthetic approaches of TeA and its derivatives have been successfully developed. This paper will mainly focus on new developments regarding TeA's herbicidal activity, mode of action, biosynthesis and chemical synthesis, and characterization of new derivatives.

1. Introduction

Tetramic acid based on the core structural unit of pyrrolidine, is a common motif among natural products (Fig. 1a). Because of their wide variety of bioactivities, naturally occurring tetramic acid derivatives have attracted interest from chemists, physicians and biologists [1–5]. Many tetramic acid derivatives have been patented as antimicrobial compounds [6–8], pesticides [9–11], and medical agents [12–13].

As one of the best characterized tetramic acid natural products, tenuazonic acid (TeA, (5*S*)-3-acetyl-5[(2*S*)-butan-2-yl]-4-hydroxy-1,5-dihydro-1*H*-pyrrol-2-one, CAS Registry No. 610-88-8, Fig. 1b) has been isolated from several phytopathogenic fungal species including *Phoma sorghina*, *Magnaporthe oryzae*, *Aspergillus* spp. and *Alternaria* spp., particularly from *Alternaria alternata*, *A. longipes and A. tenuissima* [14–20]. TeA is the most toxic of the *Alternaria* toxins. Since it was first isolated in 1957 from culture filtrates of *A. tenuis* [21], TeA has also been found in many plant materials, such as olives, cotton (seeds and bolls), sunflower seeds, peppers, tobacco seeds, sorghum kernels, rice, wheat, barley and oats as well as some fruits including apples, tomatoes, blueberries, lemons and oranges [17,22–23]. For a long time, most studies on TeA just focused on its sources, toxicity to animals, and pharmaceutical activities (e.g. antitumor, antiviral and antibiotic properties). Davies and his co-workers reported that TeA is toxic to chicken embryos and can cause haemorrhage and death in mice [24]. However, TeA also possesses antitumor and protective potential against polycyclic aromatic hydrocarbon induced skin carcinogenesis in mice [25]. TeA seems to inhibit protein biosynthesis on ribosomes by suppressing the release of new protein in eukaryotic cells [26]. Additionally, TeA isolated from *Alternaria raphani* and *A. brassicicola* cultures is an inhibitor of the bacterium *Paenibacillus larvae* [27]. As a result of its wide distribution and possible mammalian toxicity, several countries have established regulatory limits on the TeA concentration in food and feed. To ensure that the potential health risk for humans and animals posed by TeA is kept as low as practical, fast and reliable analytical methods for detection and quantitation of TeA have been developed to routinely monitor the food and feed industries [28–29].

However, in the past two decades, a steadily increasing number of studies have paid attention to the phytotoxicity exhibited by TeA. TeA weakly inhibits the activity of *p*-hydroxyphenylpyruvate dioxygenase [30] and the elongation of seedling root and shoot [31–33]. High concentrations of TeA lead to a significant increase in the multi-nucleolus ratio of *Vicia faba* root tip cells [34]. In our laboratory, large numbers of studies about TeA have been conducted in the past 20 years to develop a new type of microbial herbicide. This review summarizes our knowledge on TeA as a potential herbicide including its herbicidal activity, mode of action, biosynthesis and chemical synthesis as well as those of its derivatives.

2. Discovery of herbicidal activity

In the 1990s, *A. alternata* was first identified as a natural enemy of croftonweed (*Ageratina adenophora*), a worldwide invasive weed. Subsequently, Qiang et al. (1999) found that both conidia and mycelia of this fungus isolated from croftonweed possessed the potential for biological control [35]. Mycelia fragments blended with culture media

* Corresponding author.
E-mail address: wrl@njau.edu.cn (S. Qiang).

http://dx.doi.org/10.1016/j.pestbp.2017.01.003
0048-3575/

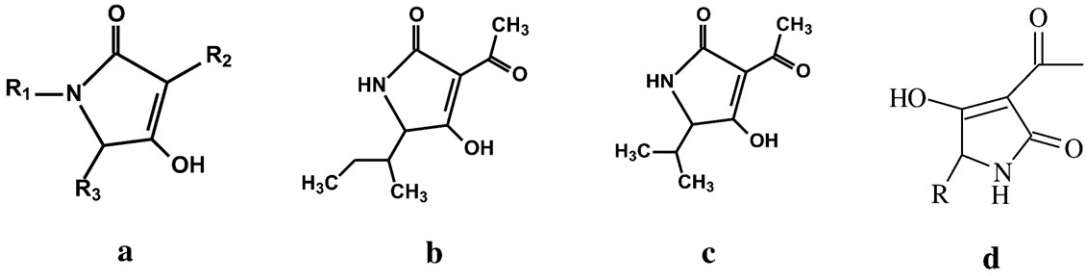

Fig. 1. The structure of tetramic acid (a), tenuazonic acid (b), 3-acetyl-5-isopropyltetramic acid (c) and derivatives of tenuazonic acid (R = $-CH_3$, $-CH_2$ (C_6H_5) or $-CH_2COOH$) (d).

were particularly effective in quickly killing the weed [35–36]. A metabolite produced by *A. alternata*, named AAC-toxin, mainly contributes this effectiveness [37–39]. The crude extract of AAC-toxin leads to ion leakage, production of reactive oxygen species (ROS), lipid peroxidation and chloroplast destruction of *A. adenophora* leaf tissues [40–41]. Bioassay results indicate AAC-toxin has toxic activity on a wide range of hosts from weed species to cultivated plants. It causes brown leaf spot and kills seedlings of mono- and dicotyledonous plants [40,42]. Field trials demonstrated high herbicidal activity of the AAC-toxin has on several species including *A. adenophora*, *Digitaria sanguinalis*, *Echinochloa crusgalli*, *Amaranthus retroflexus* and *Eclipta prostrata*. When AAC-toxin is applied post emergence at 83 ml ai/ha, >95% of large crabgrass, barnyardgrass, and redroot pigweed plants are controlled 2 days after treatment [39]. Obviously, the AAC-toxin has the potential to be developed as a new herbicide and was patented in China for this purpose [43]. Comprehensive experiments of substance isolation, purification and structure identification determined that the main active ingredient of AAC-toxin is tenuazonic acid [44–45]. Purified TeA also exhibits broad-spectrum herbicidal activity. Among 46 plant species tested, only tobacco, cotton and *Abutilon theophrasti* were tolerant to TeA [46]. TeA has also potential for controlling the serious invasive alien weed *Alternanthera philoxeroides* since it could decrease its growth, number and length of roots, and fresh weight [47]. Consequently, TeA is a suitable herbicide candidate with selectivity to tobacco and cotton, as well as for total postemergence control.

There are reports that natural TeA has acute oral toxicity with LD50 of around 200 mg/kg bodyweight [17]. On the basis of the World Health Organization Recommended Classification of Pesticides by Hazard, this means natural TeA is moderately hazardous. However, synthetic TeA was demonstrated to be only slightly hazardous to rats in an acute toxicity study. Our documents show that synthetic TeA has acute oral toxicity with LD_{50} of female and male rat of 860 and 738 mg/kg bodyweight, respectively. TeA has acute dermal toxicity with LD_{50} of greater 2000 mg/kg bodyweight and acute inhalation toxicity with LC_{50} of higher 2000 mg/m^3. Moreover, TeA did not cause either eye irritation in rabbits nor skin irritation and sensitization in Guinea pigs (data not shown). Such toxic level of TeA is considered acceptable for a postemergence herbicide. In fact, the formulation developed using whatever natural or synthetic TeA is just slightly hazardous to animal (data not shown). At >100 μg mL^{-1} concentrations, TeA inhibited the cell growth and chlorophyll content of *Chlamydomonas reinhardtii*. The micronucleus test results indicated that micronucleus frequency is >15‰ only at higher concentrations. TeA inhibited the proliferation and total protein contents of 3T3 mouse fibroblasts (3T3 cells), Chinese hamster lung cells (CHL cells) and human hepatocytes (L-O2 cells) at concentrations ranging from 12.5–400 μg mL^{-1} [48]. Field degradation experiments of TeA determined a half life of TeA of only about 3.2 days in soil and a residual period of about 20 days. Higher soil water content and temperature promotes the degradation of TeA [49].

TeA exhibits broad spectrum, rapid and high herbicidal activity as well as desirable low animal toxicity and low residuality. Thus, this mycotoxin has the potential to be developed as a herbicide. Moreover, the method of controlling weeds using TeA has been already patented in China [50] and Japan [51].

3. Mode of action

3.1. Target site of TeA

Early studies showed that TeA blocked photosystem (PS) II electron transport activity and inhibited photosynthesis, but did not affect the level and activity of RuBP carboxylases, photosynthetic pigment content, thylakoid membrane protein and PSI electron transfer chain [52–53]. The TeA concentration required to inhibit *PSII* by 50% is about 200 μM [54–55]. Studies on OJIP fast chlorophyll *a* fluorescence transients of croftonweed and spinach leaves revealed that TeA interrupts electron flow from Q_A (primary quinone acceptor) to Q_B (secondary quinone acceptor) at the *PSII* acceptor side, resulting in severe inactivation of *PSII* reaction centers (RCs). The fraction of non-Q_A and non-Q_B reducing centers had a time- and concentration- dependent linear increase. However, TeA does not affect the antenna pigments, the energy transfer from antenna pigment molecules to RCs, or the oxygen-evolving complex at the donor side of *PSII* [54,56]. Competitive displacement experiments between non-labeled TeA and [^{14}C]atrazine demonstrated that TeA has an action similar to the classical *PSII* triazine herbicide binding to the Q_B-site since atrazine binding to Q_B-site could be prevented by TeA. This means TeA interrupts *PSII* electron transport from Q_A to Q_B by competing with Q_B for the Q_B-niche in the D1 protein. However, the double-reciprocal plots of the binding of [^{14}C]atrazine in the presence of various TeA concentrations indicate that the binding behavior of TeA is different from that of the triazine herbicides. In other words, the binding of TeA to the Q_B-niche is dependent on different amino acid residues than those identified for other known *PSII* herbicides [54–55].

D1-mutants of *C. reinhardtii* were used to determine the amino acid residues involved in TeA binding. A change of amino acid at 256 position confers about 37 fold resistance to TeA, while D1-Ser264Ala and D1-Phe255Tyr mutants have approximately 8 and 2 fold resistance of TeA, respectively. Moreover, the ability of [^{14}C]atrazine to compete for TeA is weaker in the thylakoids of the D1-Gly256Asp mutant than the wild type. Therefore the 256 amino acid plays a key role in the binding of TeA to the Q_B-niche [54]. Additionally, TeA also can inhibit the activity of chloroplastic ATPase and Fd-NADP$^+$ reductase (FNR) [57].

The chemical structure of TeA shares a common characteristic group N-C = X (where X is N or O) with the classical *PSII* herbicides. The protein binding environment for *PSII* herbicides overlaps with that for Q_B. D1-H215 and D1-S264 are likely to provide hydrogen bonds to the carbonyl and amide groups of diuron, respectively [58]. However, it is suggested that classical *PSII* herbicides (diuron, atrazine and terbutryn) orient themselves preferentially towards Ser264 of the D1 protein, and the binding of phenolic herbicides (e.g. ioxynil and dinoseb) occurs via His215 [59–60]. Bioassays of TeA and its synthetic derivatives, which differ only in side chain at the 5-position, suggests that the pyrrole ring containing N—C═O group is a core part for photosynthetic inhibiting activity. Furthermore, there is an important relationship between

biological activity and the character of the side chain in the 5-position of the TeA derivatives. The presence of the hydrophobic group (alkyl side chain) in the 5-position for TeA and its derivatives is important for their high inhibitory potency. The photosynthetic inhibiting activity increases with increasing length of the side chain [54,61].

A molecular interaction model built on the basis of these findings and a previous model [54,58,62] proposes that TeA is located in the Q_B binding niche, which is a hydrophobic pocket formed by D1 residues from Phe211 to Leu275 and D2 residues from Glu219 to Ala260. TeA head group (pyrrolidone ring) binds in the pocket and the butyl side chain at 5-position is fixed hydrophobic environment in the lumen under the pocket. In the interaction between TeA and the Q_B-site, D1-G256 residue may provide a hydrogen bond with the carboxyl in 2-position of TeA thus also playing important role where as D1-S264 and D1-F255 residues may be only of marginal importance; then D1-V219 and D1-L275 residues may not be necessary at all [54]. It is clear that TeA and its derivative 3-acyl-5-alkyltetramic acids provide a novel structural framework of a potential group of photosynthesis inhibitors.

3.2. Action mechanism of TeA-induced plant leaf necrosis

In plant cells photoinhibitory damage of *PSII* can result in the formation ROS [63–64]. Histochemical analyses showed the accumulation of high levels of H_2O_2 and $O_2\bullet^-$ in cottonweeds leaf tissues within 6 h after treatment with 250 μM TeA. Based on laser-scanning confocal microscopy and subcellular localization experiments with $CeCl_3$ staining, the primary site of TeA-induced ROS generation was located to the chloroplasts of mesophyll cells. Electron Spin Resonance and ROS scavenger experiments also revealed that TeA-induced ROS produced in chloroplasts include $^1O_2$, •OH, $O_2\bullet^-$ and H_2O_2 [57]. This also differentiates TeA from classical *PSII* herbicides that mainly induce $^1O_2$ production in chloroplasts [65]. Interestingly, the PSI herbicide paraquat exerts its cellular toxicity by generating $O_2\bullet^-$ as well as •OH in chloroplasts [66]. The initial generation of TeA-induced ROS is restricted to chloroplasts and is not accompanied by visible oxidative damage to other cellular organelles or compartments. Four hours later, abundant ROS are dispersed throughout whole cells and cellular compartments, causing irreversible cellular damage such as chlorophyll breakdown, lipid peroxidation, plasma membrane rupture, chromatin condensation, DNA cleavage, and organelle disintegration that finally result in rapid cell destruction and leaf necrosis. Thus TeA-induced plant cell necrosis is a result of direct oxidative damage from the chloroplast-derived ROS burst, resulting from electron leakage and charge recombination in *PSII* as well as thylakoid over-energization due to inhibition of the *PSII* electron transport beyond Q_A and the reduction of end acceptors on the PSI acceptor side and chloroplast ATPase activity [57].

Recent studies indicate that TeA-induced $^1O_2$ activates a signaling pathway that depend on the two EXECUTER (EX) proteins, EX1 and EX2 and triggered a programmed cell death response. In *Arabidopsis* seedlings treated with TeA at half-inhibition concentration $^1O_2$-mediated and EX-dependent signaling is activated as indicated by the rapid and transient up-regulation of $^1O_2$-responsive genes in the wild type and its suppression in *ex1*/*ex2* mutants. Lesion formation only occurs when seedlings are exposed to higher concentrations of TeA for three days. In this case, the programmed cell death response triggered by $^1O_2$-mediated and EX-dependent signaling is superimposed by other events that also contribute to lesion formation [67]. This is important to further understand the mechanism of interaction between the TeA mycotoxin and its host plants. The research is also helpful to promote the commercial process of TeA as a new herbicide.

4. Biosynthesis of TeA

Tetramic acids are typical hybrid secondary metabolites originating from polyketide and α-amino acid precursors that are built up and connected by the concerted actions of polyketide synthases (PKSs) and non-ribosomal peptide synthetases (NRPSs) [3]. There are two types of fungal PKSs. One is an iterative type I PKSs, consisting of multiple catalytic domains that contain ketosynthase (KS), acyltransferase (AT) and acyl carrier protein (ACP) main domains, along with several optional β-keto processing domains, such as β-ketoacyl reductase, dehydratase and trans-acting enoyl reductase domains in a single enzyme. The other one is type III PKSs, consisting of a homodimeric KS [68–70]. PKSs recognize and use acetyl-CoA as a starter unit derived from primary metabolic pools to synthesize polyketide [71]. The NRPS portion consists of adenylation, thiolation, condensation and terminal release or cyclization domains, which is in charge of synthesis of non-ribosomal peptides [72]. In general, fungal tetramic acids are assembled by PKS-NRPS hybrid enzymes, whose structure consists of an iterative PKS followed by a single module NRPS [3]. With the rapid development of fungal whole-genome projects, additional secondary metabolite genes including a high number of *PKS* and *NPRS* genes have been identified [73–76]. This will provide further insight into tetramic acid biosynthesis and the mechanisms that regulate their production in fungi.

As a well-known tetramic acid, TeA is also expected to be a product of a PKS-NRPS hybrid enzyme [77]. In fact, TeA has been thought to be a hybrid of an isoleucine and two acetate molecules in *A. tenuis* based on feeding experiments with radioactive precursors [78–79]. The TeA biosynthetic gene from *M. oryzae* was first identified by finding two TeA-inducing conditions of a low-producing strain. TeA is synthesized from isoleucine and acetoacetyl-coenzyme A by TeA synthetase 1 (TAS1), which is a unique NRPS-PKS hybrid enzyme that begins with an NRPS module. The PKS portion of TAS1 has only a KS domain and this domain is indispensable for TAS1 activity. Phylogenetic analysis classifies this KS domain as an independent clade close to type I PKS KS domain, conducting the final cyclization step for TeA release [80]. TAS1 is the first reported fungal NRPS-PKS enzyme thus far. This may explain why the biosynthetic gene of mycotoxin TeA has remained unknown for so long. Current knowledge still limits our understanding of the mechanism of PKS-NPRS in TeA biosynthesis in different fungus species.

5. Chemical synthesis of TeA and its derivatives

The chemical structure of TeA suggests that the tetramic acid core (pyrrolidinedione) originates from a variety of amino acids, typically displaying chirality at the C5 position. Some representative chemical synthetic methods of tetramic acid have been listed in several reviews [1,3,5,81]. Schobert et al. (2004) first reported the use P-ylide reactions of polymer-bound $Ph_3P{=}C{=}C{=}O$ to quickly synthesize TeA in the laboratory. Briefly, polystyrene-bound cumulated ylide Ph_3PCCO is prepared on a large scale in two steps. It reacts with Grignard compounds, amines and alcohols to give immobilized acyl, amide and ester ylides, respectively. Their Wittig reactions lead to alkenes free of phosphane oxide. Optically pure 5-substituted tetramates are obtained from reactions of resin-bound Ph_3PCCO with α-ammonium esters in one step. TeA is accordingly prepared in just three steps [82]. Nevertheless, the approach could not be applied in industry production of TeA because of the unavoidable environmental pollution associated with P-ylide reactions.

Yang et al. (2008) provides a new strategy for the synthesis of TeA and iso-TeA (3-acetyl-4-hydroxy-5-isobutyl-1,5-dihydro-1*H*-pyrrol-2-one) using L-isoleucine and leucine as starting materials under mild reaction conditions [83]. The entire synthesis procedure includes five steps, esterification by alcohol, neutralization by sodium alcoholate, acidylation by diketene, cyclization and acidification, which are finished continuously in the presence of sodium alcoholate in a single reaction vessel. The intermediate product is directly used without further purification (Fig. 2). Furthermore, this approach does not require highly functionalized starting materials and harsh heating conditions, and is also safe and suitable for the large scale production of TeA of high quality in large quantities. Indeed based on this method TeA and a series of its

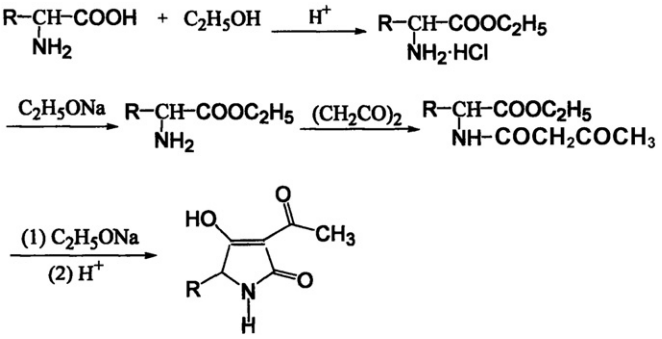

Fig. 2. Synthetic route of TeA using natural L-isoleucine as initial material. Tenuazonic acid (R group is sec-butyl), isotenuazonic acid (R group is isobutyl) [83].

derivatives with stable bioactivity were synthesized using different amino acids as parent materials [84–88].

According to the molecular interaction modeling between TeA and target D1 protein, a number of 3-acetyl-tetramic acids were designed and synthesized by modification of the TeA's side chain group at 5-position. These compounds have remarkably inhibitory activity on *PSII* electron transport and considerable herbicidal activity [61]. For example, 3-acetyl-5-isopropyltetramic acid (3-AIPTA) (Fig. 1c) synthesized from l-valine as precursor, differs only in the 5-position side chain, where 3-AIPTA has an isopropyl group while TeA has a sec-butyl group. Both 3-AIPTA and TeA share the same target site and lethal mechanism on weeds [89–90] although 3-AIPTA has weaker herbicidal activity. Another TeA-analog with a single carbon alkyl side chain at the 5 position (Fig. 1d) has even less phytotoxic activity than 3-AIPTA. This is attributed to its shorter side chain at 5-position [54]. Conversely, TeA-derivatives with a phenyl side chain (Fig. 1d) or hydrogen-group (Fig. 1d) at 5-position did not have inhibiting activity on plant growth and photosynthesis. It is probable that the phenyl group is too large to fit into the pocket of D1 and that the hydrophilic side chain is not suitable to such hydrophobic pocket [54].

6. Summary and outlook

The mycotoxin TeA is a broad-spectrum and effective *PSII* herbicide agent. Recently, a TeA micro-emulsion at 25% formulation was successfully developed and granted a Certificate of Pesticide Field Trail by the Institute for the Control of Agrochemicals, Ministry of Agriculture, P.R. China (ICAMA). Field performance supports TeA as a potential bioherbicide. However, several challenges must be overcome for its successful commercialization. First, TeA easily changes conformation under different environmental pH, temperature and medium solution, which will significantly affect its herbicidal activity. To date, this problem with TeA is still insurmountable. Second, methods and procedures should be designed and optimized for an economically viable production of TeA. Previous investigations indicate that biosynthesis by liquid fermentation is the optimal choice to produce high bioactive TeA, but its yield remains too low for industrial application. Research is underway focusing on TeA biosynthesis pathway and toxin-producing related genes of *A. alternata* to create high-yielding toxin engineered strains. Although high yield TeA synthesis can be achieved directly using L-isoleucine as the starting material, the current cost of L-isoleucine precludes its implementation. Moreover, the herbicidal activity of synthetic TeA is half lower relative to natural TeA. Finally, a mandatory detection of TeA as an unavoidable contaminant in food and feed due to its health concerns prevent its commercial development as a herbicide [28–29].

Future research should aim to identify and exploit new highly active TeA derivatives with an adequate toxicological and environmental profile than can be produced at a reasonable cost. To achieve this goal, it is necessary to build a library of derivates based on the core scaffolds of natural TeA. We have found that several candidates with a longer alkyl side chain at 5-position are better suited for tight binding in D1 protein and have a higher herbicidal activity compared to TeA. Furthermore, two of these new derivatives show slighter animal toxicity than the toxicity of TeA. Thus it is possible to design and synthesize more derivatives with high herbicidal activity and low toxicity using TeA as a template that are attractive for commercialization, and finally develop new patented herbicides instead of TeA.

Acknowledgement

This work was supported by the Hi-Tech Research and Development Program of China (863 Program, 2011AA10A206, 2006AA10A214), Jiangsu Science & Technology Pillar Program (BE2014397) and Fundamental Research Funds for the Central Universities (KYZ201530). The authors thank Bernal E. Valverde (Investigación y Desarrollo en Agricultura Tropical, Costa Rica) for helpful comments and improving the manuscript.

References

[1] B.J.L. Royles, Naturally occurring tetramic acids: structure, isolation, and synthesis, Chem. Rev. 95 (1995) 1981–2001.
[2] R. Schobert, Domino syntheses of bioactive tetronic and tetramic acids, Naturwissenschaften 94 (2007) 1–11.
[3] R. Schobert, A. Schlenk, Tetramic and tetronic acids: an update on new derivatives and biological aspects, Bioorg. Med. Chem. 16 (2008) 4203–4221.
[4] H. Zhao, Z. Cui, Y. Gu, Y. Liu, Q. Wang, The phytotoxicity of natural tetramic acid derivatives, Pest Manag. Sci. 67 (2011) 1059–1061.
[5] X. Mo, Q. Li, J. Ju, Naturally occurring tetramic acid products: isolation, structure elucidation and biological activity, RSC Adv. 4 (2014) 50566–50593.
[6] Z. Sato, S. Okuda, S. Nozoe, S. Iwasaki, H. Muro, Tetramic acid derivatives as antimicrobial agents for diseases associated with rice plants, Japan Patent, 1973, JP533658.
[7] K. Matsuo, I Kitaguchi, Y. Takata, K. Tanaka, Structure-activity relationships in tetramic acids and their copper(II) complexes, Japan Patent, 1981, JP83871.
[8] R.E. Lee, J.G. Hurdle, R. Yendapally, Analogs of tetramic acid, United States Patent, 2009, US2009/0069406A1.
[9] R. Fischer, T. Bretschneider, H.J. Santel, K. Lürrsen, R.R. Schmidt, C. Erdelen, Herbicidal and insecticidal 3-benzoyl-prrrolidin-2,4-dion-derivates, PCT, 1994, WO1994/001401.
[10] R. Fischer, S. Lehr, D. Feucht, P. Löesel, O. Malsam, G. Bojak, T. Auler, M.J. Hills, H. Kehne, C.H. Rosinger, 2-Ethyl-4,6-dimethyl-phenyl-substituted tetramic acid derivates as pest control agents and/or herbicides, PCT, 2005, WO2005/048710.
[11] F. Lieb, H. Hagemann, A. Widdig, M. Ruther, R. Fisher, T. Bretschneider, C. Erdelen, U. Wachendorff-Neumann, P. Dahmen, M. Dollinger, H.J. Santel, A. Graff, W. Andersch, N. Mencke, A. Turberg, Alkyl dihalogenated phenyl-substituted ketoenols useful as pesticides and herbicides, United States Patent, 2001, US6316486B1.
[12] H. Kawai, K. Okano, Y. Adachi, T. Matsumoto, Y. Sugawara, Y. Miyamoto, Ketone derivatives and medical application thereof, United States Patent, 2001, US6215016B1.
[13] T. Godel, H. Hilpert, R. Humm, M. Rogers-Evans, D. Rombach, C.M. Stahl, P. Weiss, W. Wostl, Tetronic and tetramic acids, United States Patent, 2005, US2005/0119329A1.
[14] S. Iwasaki, H. Muro, S. Nozoe, S. Okuda, Z. Sato, Isolation of 3,4-dihydro-3,4,8-trihydroxy-2(2H)-naphthalenone and tenuazonic acid from *Pyricularia oryzae* Cavara, Tetrahedron Lett. 1 (1972) 13–16.
[15] P.S. Steyn, C.J. Rabie, Characterization of magnesium and calcium tenuazonate from *Phoma sorghina*, Phytochemistry 15 (1976) 1977–1979.
[16] S. Nishimura, K. Kohmoto, Host-specific toxins and chemical structures from *Alternaria* species, Annu. Rev. Phytopathol. 21 (1983) 87–116.
[17] N. Montemurro, A. Visconti, *Alternaria* metabolites-chemical and biological data, in: J. Chelkowski, A. Visconti (Eds.), *Alternaria* Biology, Plant Diseases and Metabolites, Elsevier Science, Amsterdam-London-New York-Tokyo 1992, pp. 449–541.
[18] D.J. Ebbole, *Magnaporthe* as a model for understanding host-pathogen interactions, Annu. Rev. Phytopathol. 45 (2007) 437–456.
[19] V. Ostry, *Alternaria* mycotoxins: an overview of chemical characterization, producers, toxicity, analysis and occurrence in foodstuffs, World Mycotoxin J. 1 (2008) 175–188.
[20] J. Lou, L. Fu, Y. Peng, L. Zhou, Metabolites from *Alternaria* Fungi and their bioactivities, Molecules 18 (2013) 5891–5935.
[21] B.T. Rosett, R.H. Sankhala, C.E. Stickings, M.E.U. Taylor, R. Thomas, Studies in the biochemistry of micro-organisms. Metabolites of *Alternaria tenuis* auct: culture filtrate products, Biochem. J. 67 (1957) 390–400.
[22] R.A. Meronuck, J.A. Steele, C.J. Mirocha, C.M. Christensen, Tenuazonic acid, a toxin produced by *Alternaria alternata*, Appl. Microbiol. 23 (1972) 613–617.
[23] S.D. Motta, L.M.V. Soares, Simultaneous determination of tenuazonic and cyclopiazonic acids in tomato products, Food Chem. 71 (2000) 111–116.
[24] N.D. Davies, U.L. Diner, G. Morgan-Jones, Tenuazonic acid production by *Alternaria alternata* and *Alternaria tenuissima* isolated from cotton, Appl. Environ. Microbiol. 34 (1977) 155–157.

[25] M. Antony, Y. Shukla, K.K. Janardhanan, Protective effect of tenuazonic acid against dimethyl benz(a)antracene-induced skin carcinogenesis in mice, Teratog. Carcinog. Mutagen. 22 (2002) 309–314.

[26] M.A. Friedman, V. Aggarwal, G.E. Lester, Inhibition of epidermal DNA synthesis by cycloheximide and other inhibitors of protein synthesis, Res. Commun. Chem. Pathol. Pharmacol. 11 (1975) 311–318.

[27] G.L. Gallardo, N.I. Peña, P. Chacana, H.R. Terzolo, G.M. Cabrera, L-Tenuazonic acid, a new inhibitor of *Paenibacillus larva*, World J. Microbiol. Biotechnol. 20 (2004) 609–612.

[28] L. Lohrey, S. Marschik, B. Cramer, H.U. Humpf, Large-scale synthesis of isotopically labeled $^{13}C_2$-tenuazonic acid and development of a rapid HPLC-MS/MS method for the analysis of tenuazonic acid in tomato and pepper products, J. Agric. Food Chem. 61 (2013) 114–120.

[29] S. Hickert, I. Krug, B. Cramer, H.U. Humpf, Detection and quantitative analysis of the non-cytotoxic *allo*-tenuazonic acid in tomato products by stable isotope dilution HPLC-MS/MS, J. Agric. Food Chem. 63 (2015) 10879–10884.

[30] G. Meazza, B.E. Scheffler, M.R. Tellez, A.M. Rimando, J.G. Romagni, S.O. Duke, D. Nanayakkara, I.A. Khan, E.A. Abourashed, F.E. Dayan, The inhibitory activity of natural products on plant p-hydroxyphenylpyruvate dioxygenase, Phytochemistry 59 (2002) 281–288.

[31] M.C. Zonno, M. Vurro, Effect of fungal toxins on germination of *Striga hermonthica* seeds, Weed Res. 39 (1999) 15–20.

[32] E.C. Marfori, S.I. Kajiyama, E.I. Fukusaki, A. Kobayashi, Phytotoxicity of the tetramic acid metabolite trichosetin, Photochemistry 62 (2003) 715–721.

[33] K. Tylkowska, J. Grabarkiewicz-Szczesna, H. Iwanowska, Production of toxins by *Alternaria alternata* and *A. radicina* and their effects on germination of carrot seeds, Seed Sci. Technol. 31 (2003) 309–316.

[34] B. Zhou, S. Qiang, Effect of tenuazonic acid produced by *Alternaria alternata* on micronucleus and karyokinesis of *Vicia faba* root tip cells, Chin. J. App. Environ. Biol. 13 (2007) 803–806.

[35] S. Qiang, Z. Wan, Y. Dong, Y. Li, Phytotoxicity of metabolites of *Alternaria alternata* to Croften weed, in: D.C. Sun (Ed.), Sustainable Weed Management Towards the 21 Century in China, Guangxi Nationality Press, Nanning, China 1999, pp. 158–165.

[36] S. Qiang, Y. Zhu, B.A. Summerell, Y. Li, Mycelium of *Alternaria alternata* as a potential biological control agent for *Eupatorium adenophorum*, Biocontrol Sci. Techn. 16 (2006) 653–668.

[37] Z. Wan, S. Qiang, Y. Wu, Separation and activity determination of *Alternaria alternata* toxin, J. Beihua Univ. (Nat. Sci.) 2 (2001) 428–430.

[38] Z. Wan, G. Zhou, S. Qiang, Screening of toxin-producing isolates and culture medium of *Alternaria alternata*, J. Hubei Inst. Nationalities (Nat. Sci.) 19 (2001) 7–10.

[39] S. Qiang, L. Wang, R. Wei, B. Zhou, S. Chen, Y. Zhu, Y. Dong, C. An, Bioassay of the herbicidal activity of AAC-toxin produced by *Alternaria alternata* isolated from *Ageratina adenophora*, Weed Technol. 24 (2010) 197–201.

[40] Z. Wan, J. Zhu, S. Qiang, The pathogenic mechanism of toxin of *Alternaria alternata* (Fr.) Keissler to *Eupatorium adenophorum*, J. Plant Resour. Environ. 10 (2001) 47–50.

[41] Y. Chang, X. Wang, S. Qiang, Effect of *Alternaria alternata* toxin on ultrastructure of leaf tissue of *Eupatorium adenophorum* Sprengel, J. Chin. Electron Microsc. Soc. 23 (2004) 566–570.

[42] Z. Wan, S. Qiang, S. Xu, Z. Shen, Y. Dong, Culture conditions for production of phytotoxin by *Alternaria alternata* and plant range of toxicity, Chin J. Biol. Contr. 17 (2001) 10–15.

[43] S. Qiang, X. Wan, Y. Dong, Y. Li, C. Zhu, B. Dai, Biological control of weeds using the crude extract of metabolite from *Alternaria alternata* (Fr.) Keissler, China Patent, 2003, ZL00112560.5.

[44] C. An, On Potential of Metabolite of *Alternaria alternata* as Bio-Source Herbicide, in: Thesis for Master Degree, Nanjing Agricultural University, 2003.

[45] B. Zhou, C. An, Y. Dong, S. Qiang, Isolation of *Alternaria alternata* toxin using macroporous resins, J. Zhejiang Forestry College 24 (2007) 198–202.

[46] S. Chen, Y. Kang, M. Zhang, X. Wang, R.J. Strasser, B. Zhou, S. Qiang, Differential sensitivity to the potential bioherbicide tenuazonic acid probed by the JIP-test based on fast chlorophyll fluorescence kinetics, Environ. Exp. Bot. 112 (2015) 1–15.

[47] B. Zhou, X. Yan, J. Zhong, P. Jiang, S. Qiang, Pathogenicity of tenuazonic acid from *Alternaria alternata* on *Alternanthera philoxeroides*, Acta Agriculturae Zhejianggensis 22 (2010) 215–219.

[48] B. Zhou, S. Qiang, Environmental, genetic and cellular toxicity of tenuazonic acid isolated from *Alternaira alternata*, Afr. J. Biotechnol. 7 (2008) 1151–1156.

[49] B. Zhou, S. Qiang, Degradation of tenuazonic acid from *Alternaria alternata* in soil, J. Agro-Environ. Sci. 26 (2007) 572–576.

[50] S. Qiang, Y. Dong, C. An, B. Zhou, Y. Zhu, S. Chen, X. Dai, B. Dai, J. Cai, Biological control of weed using tenuazonic acid and isotenuazonic acid as well as their salts, China Patent, 2008, ZL200510038263.2.

[51] S. Qiang, Y. Dong, C. An, B. Zhou, Y. Zhu, S. Chen, X. Dai, B. Dai, J. Cai, Biological control of weed using the metabolites of *Alternaria alternata*, Japan Patent, 2012, JP4982384.

[52] X. Dai, S. Chen, S. Qiang, C. An, R. Zhang, Effect of toxin from *Alternaria alternata* (Fr.) Keissler on leaf photosynthesis of *Eupatorium adenophorum* Spreng, Acta Phytopathologica Sinica 34 (2004) 55–60.

[53] S. Chen, X. Dai, S. Qiang, Y. Tang, Effect of a nonhost-selective toxin from *Alternaria alternate* on chloroplast-electron transfer activity in *Eupatorium adenophorum*, Plant Pathol. 54 (2005) 671–677.

[54] S. Chen, X. Xu, X. Dai, C. Yang, S. Qiang, Identification of tenuazonic acid as a novel type of natural photosystem II inhibitor binding in Q_B-site of *Chlamydomonas reinhardtii*, Biochim. Biophys. Acta 1767 (2007) 306–318.

[55] S. Chen, C. Yin, X. Dai, S. Qiang, X. Xu, Action of tenuazonic acid, a natural phytotoxin, on photosystem II of spinach, Environ. Exp. Bot. 62 (2008) 279–289.

[56] S. Chen, R.J. Strasser, S. Qiang, In vivo assessment of effect of phytotoxin tenuazonic acid on *PSII* reaction centers, Plant Physiol. Biochem. 84 (2014) 10–21.

[57] S. Chen, C. Ying, S. Qiang, F. Zhou, X. Dai, Chloroplastic oxidative burst induced by tenuazonic acid, a natural photosynthesis inhibitor, triggers cell necrosis in *Eupatorium adenophorum* Spreng, Biochim. Biophys. Acta 1797 (2010) 391–405.

[58] J. Xiong, S. Subramaniam, Govindjee, Modeling of the D1/D2 proteins and cofactors of the photosystem II reaction center: implications for herbicide and bicarbonate binding, Protein Sci. 5 (1996) 2054–2073.

[59] W. Oettmeier, Herbicide resistance and supersensitivity in photosystem II, CMLS, Cell. Mol. Life Sci. 55 (1999) 1255–1277.

[60] W. Oettmeier, K. Masson, H.J. Hecht, Heterocyclic ortho-quinones, a novel type of photosystem II inhibitors, Biochim. Biophys. Acta 1504 (2001) 346–351.

[61] S. Qiang, S. Chen, C. Yang, X. Dai, Y. Dong, Method for eradicating weeds with derivatives of 3-acetyl-5-sec-butyl-4-hydroxy-3-pyrrolin-2-one, US Patent, 2014, US8921274B2.

[62] J. Xiong, S. Subramaniam, Govindjee, A knowledge-based three dimensional model of the photosystem II reaction center of *Chlamydomonas reinhardtii*, Photosynth. Res. 56 (1998) 229–254.

[63] K. Apel, H. Hirt, Reactive oxygen species: metabolism, oxidative stress, and signal transduction, Annu. Rev. Plant Biol. 55 (2004) 373–399.

[64] C. Laloi, K. Apel, A. Danon, Reactive oxygen signaling: the latest news, Curr. Opin. Plant Biol. 7 (2004) 323–328.

[65] A.W. Rutherford, A. Krieger-Liszkay, Herbicide-induced oxidative stress in photosystem II, Trends Biochem. Sci. 26 (2001) 648–653.

[66] C.F. Babbs, J.A. Pham, R.C. Coolbaugh, Lethal hydroxyl radical production in paraquat-treated plants, Plant Physiol. 90 (1989) 1267–1270.

[67] S. Chen, C. Kim, J.M. Lee, H.A. Lee, Z. Fei, L. Wang, K. Apel, Blocking the Q_B-binding site of photosystem II by tenuazonic acid, a non-host-specific toxin of *Alternaria alternata*, activates singlet oxygen-mediated and EXECUTER-dependent signaling in *Arabidopsis*, Plant Cell Environ. 38 (2015) 1069–1080.

[68] M.A. Fischbach, C.T. Walsh, Assembly-line enzymology for polyketide and nonribosomal peptide. Antibiotics: logic, machinery, and mechanisms, Chem. Rev. 106 (2006) 3468–3496.

[69] M. Hashimoto, T. Nonaka, I. Fujii, Fungal type III polyketide synthases, Nat. Prod. Rep. 31 (2012) 1306–1317.

[70] A. Gallo, M. Ferrara, G. Perrone, Phylogenetic study of polyketide synthases and nonribosomal peptide synthetases involved in the biosynthesis of mycotoxins, Toxins (Basel) 5 (2013) 717–742.

[71] J. Staunton, K.J. Weissman, Polyketide biosynthesis: a millennium review, Nat. Prod. Rep. 18 (2001) 380–416.

[72] C. Hertweck, A. Luzhetskyy, Y. Rebets, A. Bechthold, Type II polyketide synthase: gaining a deeper insight into enzymatic teamwork, Nat. Prod. Rep. 24 (2007) 162–190.

[73] H.X. Dang, B. Pryor, T. Peever, C.B. Lawrence, The *Alternaria* genomes database: a comprehensive resource for a fungal genus comprised of saprophytes, plant pathogens, and allergenic species, BMC Genomics 16 (2015) 239.

[74] F.T. Hansen, D.M. Gardiner, E. Lysøe, P.R. Fuertes, B. Tudzynski, P. Wiemann, T.E. Sondergaard, H. Giese, D.E. Brodersen, J.L. Sørensen, An update to polyketide synthase and non-ribosomal synthetase genes and nomenclature in *Fusarium*, Fungal Genet. Biol. 75 (2015) 20–29.

[75] T. Pusztahelyi, I.J. Holb, I. Pócsi, Secondary metabolites in fungus-plant interactions, Front. Plant Sci. 6 (2015) 573.

[76] Y. Hou, X. Ma, W. Wan, N. Long, J. Zhang, Y. Tan, S. Duan, Y. Zeng, Y. Dong, Comparative genomics of pathogens causing brown spot disease of tobacco: *Alternaria longipes* and *Alternaria alternata*, PLoS One 11 (2016), e0155258. .

[77] J. Collemare, A. Billard, H.U. Böhnert, M.H. Lebrun, Biosynthesis of secondary metabolites in the rice blast fungus *Magnaporthe grisea*: the role of hybrid PKS-NRPS in pathogenicity, Mycol. Res. 112 (2008) 207–2015.

[78] C.E. Stickings, Studies in the biochemistry of micro-organisms. 106. Metabolites of *Alternaria tenuis* Auct.: the structure of tenuazonic acid, Biochem. J. 72 (1959) 332–340.

[79] C.E. Stickings, R.J. Townsend, Studies in the biochemistry of microorganisms. 108. Metabolites of *Alternaria tenuis* Auct.: the biosynthesis of tenuazonic acid, Biochem. J. 78 (1961) 412–418.

[80] C.S. Yun, T. Motoyama, H. Osada, Biosynthesis of the mycotoxin tenuazonic acid by a fungal NRPS–PKS hybrid enzyme, Nat. Commun. 6 (2015) 8758.

[81] W. Bai, C. Lu, X. Wang, Recent advances in the total synthesis of tetramic acid-containing natural products, J. Chem. 2016 (2016)http://dx.doi.org/10.1155/2016/8510278.

[82] R. Schobert, C. Jagusch, C. Melanophy, G. Mullen, Synthesis and reactions of polymer-bound Ph3P═C═C═O: a quick route to tenuazonic acid and other optically pure 5-substituted tetramates, Org. Biomol. Chem. 2 (2004) 3524–3529.

[83] C. Yang, S. Qiang, L. Huang, P. Zhang, Z. Zhu, Synthesis of tenuazonic acid and isotenuazonic acid manufacturing method, China Patent, 2008, ZL200610038765.X.

[84] Z. Zhu, Q. Shi, B. Han, X. Wang, S. Qiang, C. Yang, Synthesis, characterization and biological activities of novel (E)-3-(1-(alkyloxyamino)ethylidene)-1-alkylpyrrolidine-2,4-dione derivatives, Bull. Korean Chem. Soc. 31 (2010).

[85] Z. Zhu, X. Wang, F. Meng, Q. Li, X. Zheng, S. Qiang, C. Yang, Synthesis, characterization and biological activities of novel (Z)-3-((E)-1-(alkyloxyimino)ethyl)-5-arylidene-4-hydroxypyrroline-2-one derivatives, J. Heterocycl. Chem. 47 (2010) 1328–1334.

[86] X. Wang, T. Si, Q. Li, Z. Zhu, X. Zhu, S. Qiang, C. Yang, Synthesis, characterization and biological activity of novel (5-*RS*,6-*S*)-5-sec-butyl-3-(1-substituted-amino)ethylidene-1*H*-pyrrolidine-2,4-diones, ARKIVOC (ii) (2010) 31–48.

[87] X. Zheng, B. Han, X. Wang, S. Qiang, C. Yang, Synthesis and bioactivity of novel (Z,E)-1-(substituted phenyl)-3-[α-(alkyloxyimino)benzylidene] pyrrolidine-2,4-dione derivatives, Heterocycl. Commun. 17 (2011) 73–78.
[88] W. Xu, M. Chen, K. Wang, Z. Ren, A. Lu, C. Yang, Synthesis, characterization, and antifungal activity of phenylpyrrole-substituted tetramic acids bearing carbonates, Molecules 21 (2016) 355.
[89] S. Chen, F. Zhou, C. Yin, R.J. Strasser, S. Qiang, C. Yang, Application of fast chlorophyll *a* fluorescence kinetics to probe action target of 3-acetyl-5-isopropyltetramic acid, Environ. Exp. Bot. 71 (2011) 269–279.
[90] S. Chen, C. Yin, R.J. Strasser, Govindjee, C. Yang, S. Qiang, Reactive oxygen species from chloroplasts contribute to 3-acetyl-5-isopropyltetramic acid-induced leaf necrosis of *Arabidopsis thaliana*, Plant Physiol. Biochem. 52 (2012) 38–51.