Pesticide Biochemistry and Physiology 135 (2017) 47–51

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

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

Synthesis and antifungal activity of 2-allylphenol derivatives against fungal plant pathogens

Tianli Qu [a,b], Shumei Gao [c,d], Jianqiang Li [c,d,*], Jianjun J. Hao [e], Pingsheng Ji [b]

[a] Chemistry and Pharmacy College, Qingdao Agricultural University, Shandong 266109, China
[b] Department of Plant Pathology, Coastal Plain Experiment Station, University of Georgia, Tifton, GA 31794, USA
[c] Key Laboratory of Plant Pathology, Department of Plant Pathology, China Agricultural University, Beijing 100193, China
[d] Beijing Engineering Research Center of Seed and Plant Health, Beijing Key Laboratory of Seed Disease Testing and Control, Beijing 100193, China
[e] School of Food and Agriculture, University of Maine, Orono, ME 04469, USA

ARTICLE INFO

Article history:
Received 18 March 2016
Received in revised form 7 June 2016
Accepted 20 June 2016
Available online 21 June 2016

Keywords:
2-Allylphenol
2-(2-Hydroxypropyl) phenol
2-(3-Hydroxypropyl) phenol
Fungal plant pathogen

ABSTRACT

2-Allylphenol (2-AP) is an effective fungicide against a number of plant pathogens, which can be metabolized and bio-transformed to four chemical compounds by *Rhizoctonia cerealis*. To determine if its degradation affects antifungal activity, two major metabolites derived from 2-AP including 2-(2-hydroxypropyl) phenol and 2-(3-hydroxypropyl) phenol were synthesized. Inhibition of mycelial growth of several plant pathogens by the metabolites was evaluated, and structures of two metabolites were determined by hydrogen nuclear magnetic resonance (^{1}H NMR). Among these metabolites, only 2-(2-hydroxypropyl) phenol inhibited test pathogens effectively. EC_{50} values of 2-(2-hydroxypropyl) phenol for inhibition of mycelial growth of *R. cerealis*, *Pythium aphanidermatum*, *Valsa mali* and *Botrytis cinerea* ranged from 1.0 to 23.5 μg/ml, which were lower than the parental fungicide 2-AP that ranged from 8.2 to 48.8 μg/ml. Hyphae of *R. cerealis* and *P. aphanidermatum* treated with 2-(2-hydroxypropyl) phenol were twisted. Newly developed hyphae were slender, twisted and swollen on the tip, while old hyphae were hollow and ruptured. This is the first report indicating the formation of 2-(2-hydroxypropyl) phenol may have contributed to toxicity of 2-allylphenol in control of plant pathogens.

1. Introduction

2-Allylphenol (2-AP) is a synthetic fungicide that is a mimic to ginkgol from Gingko (*Gingko biloba* L) [1,2]. It controls a variety of fungal plant diseases effectively such as apple stem rot (*Valsa mali*), tomato gray mold (*Botrytis cinerea*), maize leaf spot (*Drechslera turcica*), wheat sharp eyespot (*Rhizoctonia cerealis*) and strawberry powdery mildew (*Sphaerotheca aphanis*) [1–5]. The mechanism responsible for the inhibition of pathogens includes inducing cyanide-resistant respiration, causing ATP decrease, and inhibiting respiration [6]. Because of its effective disease control efficacy, 2-AP has currently been widely applied in >12 million ha in China [1]. With such broad applications, biodegradation of 2-AP residues in plant products and their toxicology was studied [7,8,10].

Different methods have been used to study residues of 2-AP and its derivatives in plant-associated environments, including high performance liquid chromatography (HPLC) and indirect competitive enzyme-linked immunosorbent assay (icELISA). Studies indicated that the half-life of 2-AP (synonym: *o*-allylphenol) in strawberry was 4.4 days, and in strawberry fruit it was degraded by >90% before harvesting [5,7]. In tomato fruit, the half-life of 2-AP was 6.37 days and >80% of the compound was degraded by harvest [8]. 2-AP could be taken up by wheat seedlings and it was transported quickly from roots to the top of the seedlings [9]. In another study, transportation and distribution of 2-AP from leaf to roots in wheat seedling was determined. The results indicated that 2-AP moved from leaves to roots of wheat seedlings in 4 h [10].

Hu et al. reported that four major photoproducts of 2-AP could be detected in soil, including 2,4,5-trihydroxybenzaldehyde, *p*-allylphenol, methyl-2-hydroxphenylacetate, and 2-methyl-benzodihydrofuran [8]. A more recent study indicated that 2-AP was metabolized and biotransformed by the target pathogen *R. cerealis*, and four major metabolites of 2-AP were identified to be 2-(2-hydroxyphenyl) acetic acid, 2-(2,3-dioxypropyl) phenol, 2-(2-hydroxypropyl) phenol and 2-(3-hydroxypropyl) phenol [11]. However, no further information is available on the impact of these metabolites on plant pathogens and the environment.

* Corresponding author at: Key Laboratory of Plant Pathology, Department of Plant Pathology, China Agricultural University, Beijing 100193, China.
E-mail address: lijq231@cau.edu.cn (J. Li).

http://dx.doi.org/10.1016/j.pestbp.2016.06.006
0048-3575/

The objectives of this study were to synthesize the major metabolites of 2-allylphenol under laboratory conditions and determine antifungal property of the metabolites. Understanding activities of the metabolites in suppression of plant pathogens will help elucidate mechanisms associated with disease reduction by 2-allylphenol and have the potential to identify novel molecules for managing plant diseases.

2. Materials and methods

2.1. Chemicals

2-allylphenol (≥99% a.i.) was provided by the Institute for the Control of Agrochemicals, Ministry of Agriculture, Beijing, China. 2-(2-hydroxyphenyl) acetic acid was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Analytical grade chemicals ethyl acetate, acetone, petroleum benzin, alcohol, sulphuric acid, sodium borohydride, sodium hydroxide, anhydrous tetrahydrofuran, three boron trifluoride-ether solution (47%), hydrogen peroxide (30%), Congo red stain (1% in ethanol) and triton X-100 were purchased from Beijing Chemical Works (Beijing, China).

2.2. Synthesis of 2-(2-hydroxypropyl) phenol and 2-(3-hydroxypropyl) phenol

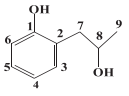
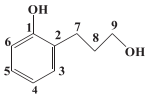
For synthesis of 2-(2-hydroxypropyl) phenol, 2-AP (99%) (13.4 g) and 20 ml ethyl acetate were put in a 250 ml 3-neck flask with a thermometer placed in and connected with a reflux condensing tube and constant voltage funnels. An aliquot of 50 ml 50% H_2SO_4 was gradually dropped into the flask with stirring in ice bath for 10 min. After H_2SO_4 was all added, the ice was removed and stirring was continued under electric heating (112 °C). This reaction mixture was stirred continuously until light brown color was observed; then cooled down to room temperature [12,13].

To synthesize 2-(3-hydroxypropyl) phenol, 2-AP (99%) (13.4 g), sodium borohydride (3.0 g) and anhydrous tetrahydrofuran (100 ml) were added in a 250 ml boiling flask-3-neck and stirred under nitrogen atmosphere at 25 °C. After 30 min, 15 ml three boron trifluoride-ether solution (47%) was added and the solution was stirred continuously at room temperature for 3 h. A volume of 20 ml of distilled water was dropped slowly to the flask for splitting excess hydride. Then 20 ml NaOH (3 mol/l) was put in the boiling flask and 20 ml hydrogen peroxide (30%) was dropped at 30 °C. The mixture was stirred continuously for 2 h. After diluting hydrochloric acid acidizing reaction liquid, the mixture was extracted using isopropyl ether for three times with anhydrous magnesium sulfate drying. The solvent was evaporated using a rotary evaporator at 35 °C [14,15].

The products were collected and purified by a gel column chromatography using petroleum benzin, ethyl acetate and alcohol (150:10:1, v/v/v) as a solvent. The compounds were analyzed and confirmed by thin layer chromatography (TLC) using silica gel plates, with petroleum benzin, ethyl acetate, and alcohol (6:1:0.1, v/v/v) as developing solvent (data not shown). As reported previously [11], mass spectrometric analysis under negative ions in the 20–400 *m/z* (mass-to-charge ratios) range indicated that 2-(2-hydroxypropyl) phenol displayed protonated molecules $[M-H]^-$ at 150.9 *m/z* and ion fragments at 107.2 and 93.3 *m/z*. 2-(3-hydroxypropyl) phenol showed protonated molecules at 150.9 *m/z* and ion fragments at 107.1 *m/z*. Molecular weight of both compounds was 152.

For further confirmation of the structure, 500 MHz ^{1}H NMR spectroscopy was performed to determine number of proton, protonic environment and different functional frequencies of the synthesized compounds. ^{1}H NMR data of 2-(2-hydroxypropyl) phenol and 2-(3-hydroxypropyl) phenol were summarized in Table 1. The ^{1}H NMR spectra of 2-(2-hydroxypropyl) phenol showed high similarity to that of 2-(3-hydroxypropyl) phenol, only some protons in the side chain of aromatic ring occurred differently. In 2-(2-hydroxypropyl) phenol, hydroxylation took place at methylene group 8, while in 2-(3-hydroxypropyl) phenol it occurred at methyl group 9. The result indicated that 2-(2-hydroxypropyl) phenol and 2-(3-hydroxypropyl) phenol were isomers. ^{1}H NMR spectral data of 2-(2-hydroxypropyl) phenol and 2-(3-hydroxypropyl) phenol agreed with published data [11,13].

Table 1
Hydrogen nuclear magnetic resonance (^{1}H NMR) parameters of 2-(2-hydroxypropyl) phenol and 2-(3-hydroxypropyl) phenol in $CDCl_3$ (500 mHz).

Position of hydrogen	2-(2-hydroxypropyl) phenol	2-(3-hydroxypropyl) phenol
Molecular structure		
Ar-H	δ7.29–6.65 (4H, m)	δ7.06–6.81 (4H, m)
7-H	δ3.26–2.76 (2H, m)	δ2.75 (2H, t. J = 8.0 Hz, J = 7.5 Hz)
8-H	δ3.94 (1H, m)	δ1.88 (2H, m)
9-H	δ1.24 (3H, d. J = 6.3 Hz)	δ 3.63 (2H,*t*. J = 6.5 Hz, J = 5.5 Hz)

Chemical shifts (δ) are expressed in parts per million (ppm). s: singlet, d: doublets, t: triplets and m: multiplets.

2.3. In vitro assays of antimicrobial activities

The inhibitory effects of three metabolites, 2-(2-hydroxypropyl) phenol, 2-(3-hydroxypropyl) phenol and 2-(2-hydroxyphenyl) acetic acid, as well as the parent compound 2-AP, were assessed against plant pathogenic fungi including *Pythium aphanidermatum*, *Valsa mali*, *Botrytis cinerea*, and *Rhizoctonia cerealis*. Another metabolite, 2-(2,3-dioxypropyl) phenol, was not synthesized successfully, so it was not tested. The above compounds were dissolved in methanol and used to amend potato dextrose agar (PDA) plates (9 cm), at final concentrations of 0.1, 0.5, 1, 5, 10, 25, 50 and 100 μg/ml of the compounds. An agar plug (5 mm) was removed from the edge of an actively growing culture of each pathogen and placed at the center of a PDA plate amended with the fungicides. PDA plates amended with only methanol (0.5% final concentration) were used as a control. Four replicate plates were used for each fungicide concentration for each pathogen. After incubation at 25 °C in darkness for 5 days, the radial growth of mycelia was measured. The 50% effective concentration (EC_{50}) was calculated for the compounds that showed antimicrobial activity [16]. The experiment was repeated one more time under the same conditions.

2.4. Effect of 2-(2-hydroxypropyl) phenol on mycelia of fungal pathogens

Isolates of *P. aphanidermatum*, *V. mali*, *B. cinerea* and *R. cerealis* were grown on PDA with sterile cellulose membranes placed on the surface of the plates at 25 °C in the dark for 3 days. An aliquot of 0.5 ml 2-(2-hydroxypropyl) phenol (100 μg/ml) was applied at the edge of the

Table 2
Inhibition of growth of four plant pathogens by 2-(2-hydroxypropyl) phenol.

Pathogens	Fungicides	R^2[a]	EC_{50} (μg/ml)
Pythium aphanidermatum	2-Allylphenol	0.9728	34.076
	2-(2-Hydroxypropyl) phenol	0.9842	20.316
Valsa mali	2-allylphenol	0.9917	20.149
	2-(2-Hydroxypropyl) phenol	0.9652	14.991
Botrytis cinerea	2-Allylphenol	0.9669	48.752
	2-(2-Hydroxypropyl) phenol	0.9143	23.538
Rhizoctonia cerealis	2-Allylphenol	0.8937	8.152
	2-(2-Hydroxypropyl) phenol	0.8901	1.039

[a] Square of correlation coefficient of regression: $Y = a + bX$, where Y is Probit value corresponding 50% response, X is log dose, a is intercept, b is slope.