JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Identification of a Germicidal Compound against Picornavirus in Bamboo Pyroligneous Acid

Shinsuke Marumoto,[†,∥] Seiji P. Yamamoto,[†,‡,∥] Hiroshi Nishimura,[†] Koji Onomoto,[‡,⊥] Mitsuyoshi Yatagai,[§,#] Kazufumi Yazaki,[†] Takashi Fujita,[‡] and Takashi Watanabe*[,†]

[†]Research Institute for Sustainable Humanosphere, Kyoto University, Uji, Kyoto 611-0011, Japan

[‡]Institute for Virus Research, Kyoto University, Kyoto 606-8501, Japan

[§]Institute of Wood Technology, Akita Prefectural University, 11-1 Noshiro, Akita 016-0876, Japan

ABSTRACT: The germicidal activity of pyroligneous acid (PA) against a picornavirus, encephalomyocarditis virus (EMCV), was analyzed, and the component responsible for its disinfectant activity was identified. Bamboo PA (BPA) inactivated EMCV, but neutralization of BPA abolished this activity. Using liquid−liquid phase extraction and silica gel column chromatography, the hydrophobic active fraction of BPA was separated and its 12 major components were identified. The active fraction was reconstructed by mixing synthetic chemicals at the determined concentrations, and a subtraction series of one chemical from the complete mixture was prepared. An in vitro virus assay demonstrated that phenol was the sole germicidal component, and acetic acid augmented the phenol's inactivating activity resulting in >5-log decrease in EMCV infectivity. Considering the low environmental risk of PA, these findings suggest that BPA is a potentially useful agent for preventing viral epidemics in agricultural and human environments.

KEYWORDS: *pyroligneous acid, bamboo vinegar, encephalomyocarditis virus, picornavirus, germicide*

INTRODUCTION

Pyroligneous acid (PA, also called wood vinegar or pyroligneous liquor) is the crude condensate of smoke generated during the process of making wood charcoal. Chemically, PA is a complex mixture of water, phenols, furan carboxaldehydes, pyrones, and carboxylic acids,[1−3] and it has been traditionally used as a sterilizing agent, deodorizer, fertilizer, antimicrobial, and growth-promoting agent. Additionally, it has been used as a source of smoke flavor.[4] Smoke flavor can be used in foods as an additional barrier to prevent microbial growth at levels that comply with good manufacturing practice.[5] It was reported that the antimicrobial activity of PA is attributed to the presence of compounds such as phenolic derivatives, carbonyls, and organic acids.[6]

Bamboo is a renewable bioresource when its plantation and use cycle are properly managed. Pyrolyzing bamboo under oxygen-limited conditions produces bamboo charcoal, and condensation of the smoke from pyrolysis produces bamboo PA (BPA), a brown-red transparent liquid. BPA contains more than 200 types of chemicals, among which acetic acid is the primary component.[7,8] Mu and colleagues demonstrated that BPA exerted an obvious promotional effect on germination and radicle growth for some types of seeds, e.g., lettuce.[9,10] The intriguing fact is that composition of BPA from moso bamboo (*Phyllostachys pubescens*), which is the same species used in this study, was different from that from madake bamboo (*Phyllostachys bambusoides*) despite their close relationship.[9] In addition, the temperature at the collection step of crude BPA critically affects the efficacy of BPA.[10] Regarding its effect on the regulation of germination and growth, it is known that BPA collected at temperatures of up to 250 °C promoted radicle and hypocotyl growth, whereas BPA collected at temperatures from 250 °C−400 °C inhibited their growth.[10]

As mentioned above, PA has been used as a sterilizing and antimicrobial agent. Additionally, it has also been reported that PA inactivates tobacco mosaic virus.[11] It activates against plant viruses, and a recent report has demonstrated that BPA inactivates porcine reproductive and respiratory syndrome (PRRS) virus, an enveloped RNA virus belonging to the Arteriviridae family.[12] Thus, PA has great potential for inactivating other viruses, and considering its aforementioned low environmental risk, it can be used as an atomizing agent to kill airborne viruses, such as foot-and-mouth disease virus (FMDV).

The potential germicidal ability of BPA against picornaviruses has not been systemically studied. In this study, to examine the efficacy of BPA as a germicide against picornaviruses, encephalomyocarditis virus (EMCV), which belongs to the same Picornaviridae family as FMDV, was used as a model virus because FMDV is under usage restrictions. Components responsible for the germicidal activity in BPA were first identified.

MATERIALS AND METHODS

PAs and Chemicals. For this study, BPA from moso bamboo (*P. pubescens*) and distilled BPA (dBPA) from Minobu Chikutan Kigyou Kumiai (Yamanashi, Japan) were used. BPA and dBPA contained 3.5% and 4.8% acetic acid, respectively. Furfural, 5-methylfurfural, and 2-methyl-2-cyclopenten-1-one were purchased from Sigma-Aldrich

Received: May 16, 2012
Revised: July 24, 2012
Accepted: July 31, 2012
Published: July 31, 2012

dx.doi.org/10.1021/jf3021317 | J. Agric. Food Chem. 2012, 60, 9106−9111

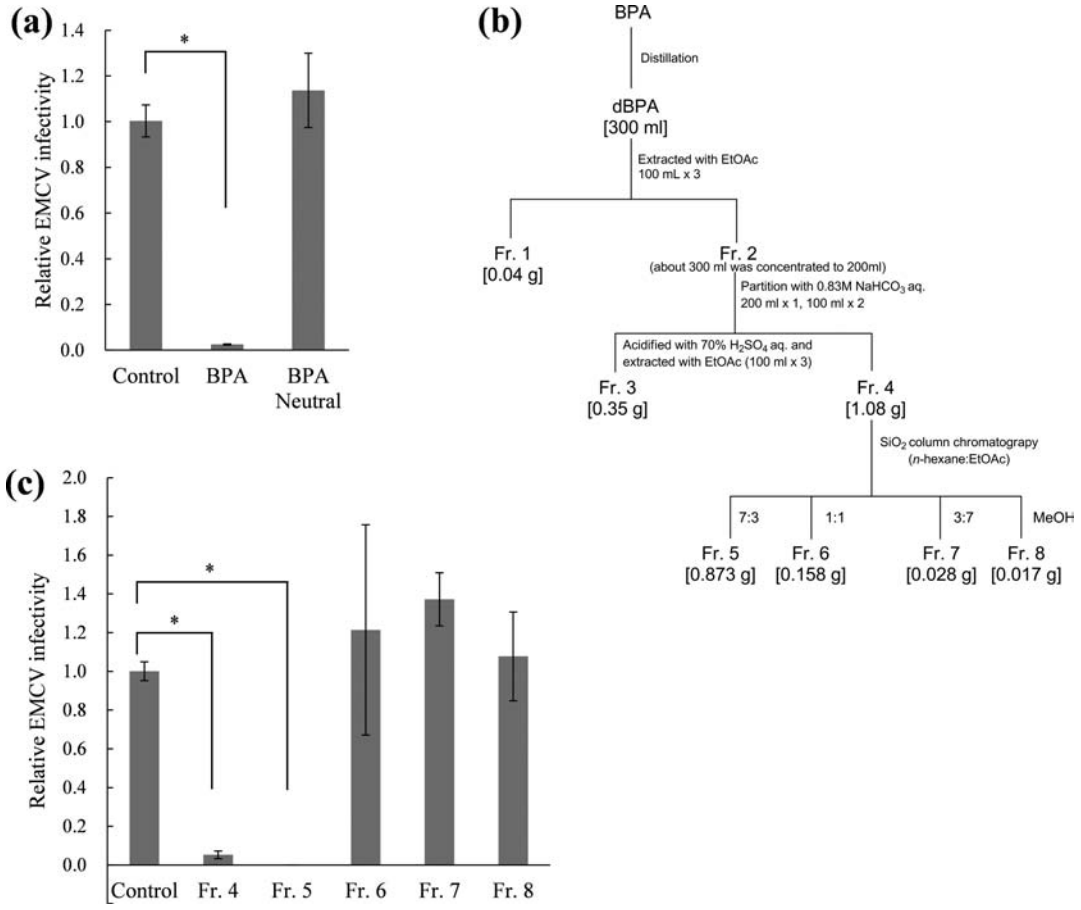

Figure 1. Fractionation and virus-inactivating ability of BPA: (a) EMCV inactivation assay using BPA. BPA and $NaHCO_3$-neutralized BPA were investigated using a viral inactivation assay and quantitative real-time PCR. Water was used as the control. (b) Schematic representation of the BPA fractionation process. (c) EMCV inactivation assay using fractionated BPA. BPA fractions (Fr.) 4−8 were investigated using a viral inactivation assay and quantitative real-time PCR. *, $P < 0.05$ by Student's *t*-test.

(Tokyo, Japan). Acetylfuran was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Phenol, *o*-, *m*-, and *p*-cresols, guaiacol, 4-ethylphenol, 4-methylguaiacol, 4-ethylguaiacol, and butylated hydroxytoluene (BHT) were purchased from Wako Pure Chemical Industry Co., Ltd. (Japan).

Fractionation of BPA. As shown in Figure 1b, dBPA (300 mL) was extracted with ethyl acetate (EtOAc; 100 mL × 3). The solvent was then evaporated to 200 mL and partitioned between 0.83 M $NaHCO_3$ aq (200 mL × 1, 100 mL × 2). The EtOAc phase was evaporated to give fraction (Fr.) 4 (1.08 g), while the aqueous layer was acidified with 70% H_2SO_4 and then extracted with EtOAc to yield acidic Fr. 3 (0.35 g). Fr. 4 was further fractionated by silica gel column chromatography (*n*-hexane/EtOAc) to give Frs. 5−8.

Gas Chromatography−Mass Spectrometry. The compounds in the fraction were identified by gas chromatography−mass spectrometry (GC-MS). The GC-MS analysis was conducted using a Shimadzu GCMS-QP5050A (Shimadzu, Co., Ltd., Kyoto, Japan) at an ionization voltage of 70 eV and an electron multipler and transfer line temperature of 280 °C on a DB-5MS column (25 m × 0.25 mm i.d., 0.25−μm film thickness; J&W Scientific). The temperature program was as follows: 40 °C for 3 min, then increased by 3.2 °C/min to 130 °C, 12 °C/min to 280 °C, and held for 3.2 min. The other parameters were as follows: injection temperature, 280 °C; ion source temperature, 280 °C; carrier gas, He at 1.5 mL/min; injection volume, 0.5 μL; split ratio, 1:10; and mass range m/z 40−600.

The identification of individual compounds was based on the comparison of their relative retention time with those of authentic samples on the DB-5MS column, and matching their mass spectra peaks with those obtained from authentic samples and spectral data from the Wiley7 and NIST107 libraries. The identified 12 components were quantified by the GCMS using BHT as an internal standard.

Cell Culture. L929 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin.

Viral Inactivation Assay. EMCV has been described previously.[13] For the inactivation assay, 10 μL of medium containing 1 × 105 pfu/mL EMCV was incubated with an equal amount of BPA samples at room temperature for 1 h. Each mixture of EMCV and BPA samples was diluted 5 times by culture medium, and 10 μL of the diluted samples was then added to 1 mL of culture medium containing 2.5 × 105 L929 cells in a 12-well plate. After 6 h of virus infection, cells were harvested and subjected to quantitative real-time PCR.

Quantitative Real-Time PCR. Total RNA was extracted from EMCV infected cells with TRIZOL (Invitrogen) and treated with DNase I (Roche). High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) was used for cDNA synthesis. Viral RNA levels were monitored with the 7500 Real-Time PCR system and Power SYBR Green PCR Master Mix (Applied Biosystems), using the following EMCV-specific primers: forward, 5′-TTA TAG -TGC-CGG-ACC-TGG-CA-3′ and reverse, 5′-CCC-AAG-CTC-CCA-GTG-TTG-TC-3′. The RNA copy number of EMCV was normalized to that of internal 18S rRNA, which was monitored using TaqMan Universal PCR Master Mix II and TaqMan primer-probe for 18S rRNA (Applied Biosystems).

Statistical Analysis. Student's *t*-test was used to determine statistical significance. $P < 0.05$ was considered significant.

dx.doi.org/10.1021/jf3021317 | J. Agric. Food Chem. 2012, 60, 9106−9111

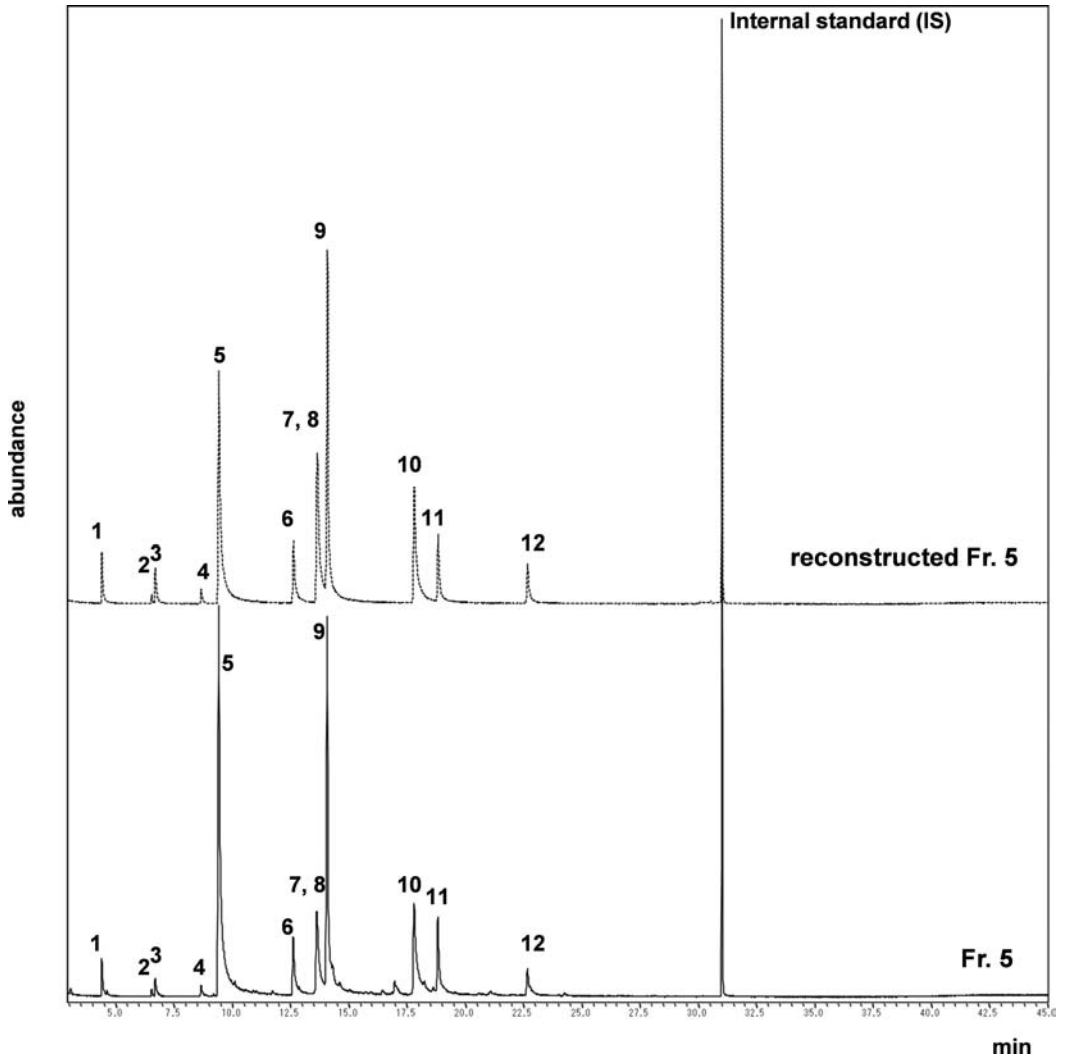

Figure 2. Total ion chromatogram of Fr. 5 and reconstructed Fr. 5 using GC-MS. The chemicals represented by numbered peaks are shown in Table 1.

RESULTS AND DISCUSSION

EMCV-Inactivating Ability of BPA. PA can often be used as a safer alternative to chemical pesticides and fungicides. PA has also been reported to inactivate tobacco mosaic virus,[11] and

Table 1. Composition of Fraction 5

no.	compds	RT (min)	relative content (%)	identification[a]
1	furfural	4.43	1.6	MS, ST
2	2-methyl-2-cyclopenten-1-one	6.56	0.3	MS, ST
3	acetylfuran	6.74	1.0	MS, ST
4	5-methylfurfural	8.69	0.6	MS, ST
5	phenol	9.45	35.5	MS, ST
6	*o*-cresol	12.65	4.3	MS, ST
7,8	*m*-and/or *p*-cresols	13.67	9.8	MS, ST
9	guaiacol	14.10	29.0	MS, ST
10	4-ethylphenol	17.83	8.9	MS, ST
11	4-methylguaiacol	18.85	6.1	MS, ST
12	4-ethylguaiacol	22.69	2.8	MS, ST
	total		100	

[a]MS, NIST, and Wiley libraries; ST, authentic standard compounds.

Table 2. Concentration of Identified Compounds in Fr. 5 and Reconstructed Fr. 5

		Fr. 5		reconstructed Fr. 5	
no.	compds	relative content (%)	concn (mg/mL)[a]	relative content (%)	concn (mg/mL)[a]
1	furfural	1.6	10.5	3.0	15.1
2	2-methyl2-cyclopenten-1-one	0.3	0.8	0.4	1.4
3	acetylfuran	1.0	6.2	2.0	8.2
4	5-methylfurfural	0.6	4.4	1.1	5.5
5	phenol	35.5	155.0	25.0	98.0
6	*o* -cresol	4.3	23.5	4.3	33.0
7, 8	*m -and/or p* -cresols	9.8	53.0	16.2	58.7
9	guaiacol	29.0	100.0	23.8	77.8
10	4-ethylphenol	8.9	47.5	15.6	52.8
11	4-methylguaiacol	6.1	32.1	5.3	35.2
12	4-ethylguaiacol	2.8	16.5	3.2	18.7
total		100.0	449.5	100.0	404.3

[a]Concentation was calculated by the internal standard nethod.

dx.doi.org/10.1021/jf3021317 | J. Agric. Food Chem. 2012, 60, 9106−9111

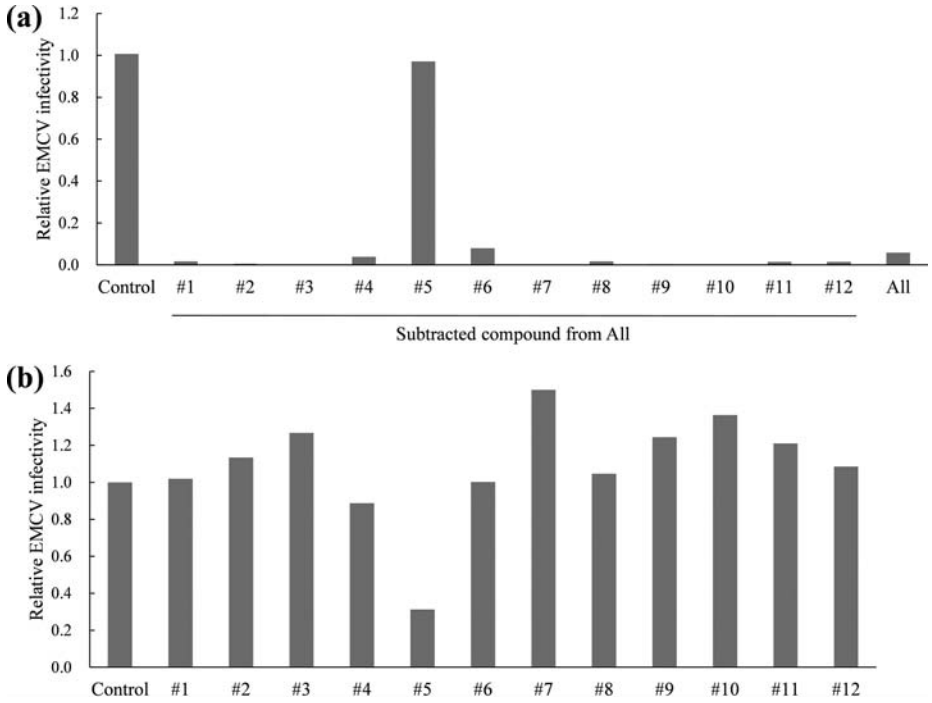

Figure 3. Identification of phenol as the EMCV-inactivating compound in Fr. 5: (a) EMCV inactivation assay using reconstructed Fr. 5. Reconstructed Fr. 5, indicated as "All," which contains 12 components as shown in Table 2, and the compounds numbered 1 through 12, which indicate the component that was subtracted from "All," were investigated using the viral inactivation assay and quantitative real-time PCR. (b) EMCV inactivation assay using each component of Fr. 5. Compounds numbered 1 through 12, which were used at the concentrations shown in Table 2, were investigated using the viral inactivation assay and quantitative real-time PCR.

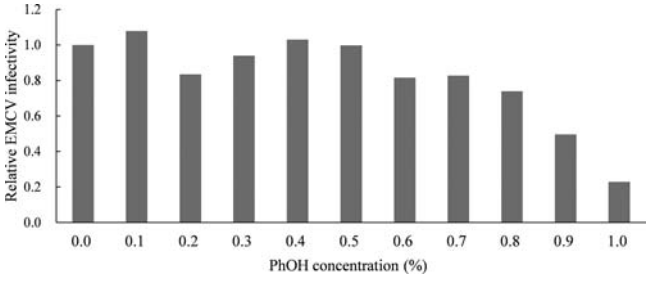

Figure 4. Effect of phenol (PhOH) on the infectivity of EMCV. Serially diluted PhOH was investigated using the viral inactivation assay and quantitative real-time PCR.

this characteristic distinguishes PA from other pesticides. Therefore, PA could be potentially useful for the inactivation of other viruses. To evaluate the efficacy of a certain PA, it should be correctly reproduced according to the definition and standards because the chemical composition of PA varies with the type of wood and production process. Hence, BPA was produced from moso bamboo (*P. pubescens*) in accordance with the guideline of the Japan Mokusaku-eki Association. The effect of BPA on EMCV infectivity was tested. EMCV, which belongs to the *Cardiovirus* genus and Picornaviridae family, is characterized by its small size (20−30 nm diameter) and resistance to lipophilic germicides because of the absence of a lipid envelope.[14] For the antivirus assay, 10 μL of medium containing EMCV was mixed with 10 μL of BPA for 1 h at room temperature, and then the mixture was added to L929 cells. The RNA level of EMCV in infected cells was measured by quantitative real-time polymerase chain reaction (PCR). As shown in Figure 1a, treatment of EMCV with BPA significantly decreased EMCV infectivity to <5%, indicating that BPA can inactivate EMCV; however, neutralized BPA did not affect the infectivity of EMCV. As it is well known that EMCV is stable between pH 3 and 9, the pH of BPA (pH 3.5) did not appear to influence EMCV infectivity. These data suggest that BPA contains compound(s) that inhibit(s) EMCV infectivity under acidic conditions.

Fractionation of BPA. EtOAc extract of BPA (Fr. 4) exhibited virus-inactivating ability, and therefore, silica gel column chromatography was applied to the EtOAc extract to fractionate BPA into Frs. 5−8 (Figure 1b). The virus-inactivating activity of each fraction was then assayed. Fr. 5 (*n*-hexane-EtOAc, 7:3) showed the strongest inhibitory activity even at neutral pH, probably because antiviral compounds were highly concentrated in Fr. 5, as observed with Fr. 4 (Figure 1c). To identify the compound(s) that can inactivate EMCV, Fr. 5 was analyzed by GC-MS. As shown in Figure 2, 12 compounds, furfural (1.6%), 2-methyl-2-cyclopenten-1-one (0.3%), acetylfuran (1.0%), 5-methylfurfural (0.6%), phenol (35.5%), *o*-cresol (4.3%), *m*- and/or *p*-cresols (9.8%), guaiacol (29.0%), 4-ethylphenol (8.9%), 4-methylguaiacol (6.1%), and 4-ethylguaiacol (2.8%), were identified and determined as major components of the fraction (Table 1).

Reconstruction of the Active Fraction. To characterize the germicidal activity and active compounds in Fr. 5, the composition of Fr. 5 was reconstructed using 12 commercially available synthetic chemicals and analyzed by GC-MS (Figure 2). The concentrations of each component in reconstructed Fr. 5 are as follows: furfural (15.1 mg/mL: by internal standard method; internal standard, butylated hydroxytoluene), 2-methyl-2-cyclopenten-1-one (1.4 mg/mL), acetylfuran (8.2 mg/mL), 5-methylfurfural (5.5 mg/mL), phenol (98.0 mg/mL), *o*-cresol (33.0 mg/mL), *m*- or/and *p*-cresols (58.7 mg/mL), guaiacol (77.8 mg/mL), 4-ethylphenol (52.8 mg/mL), 4-

dx.doi.org/10.1021/jf3021317 | J. Agric. Food Chem. 2012, 60, 9106−9111

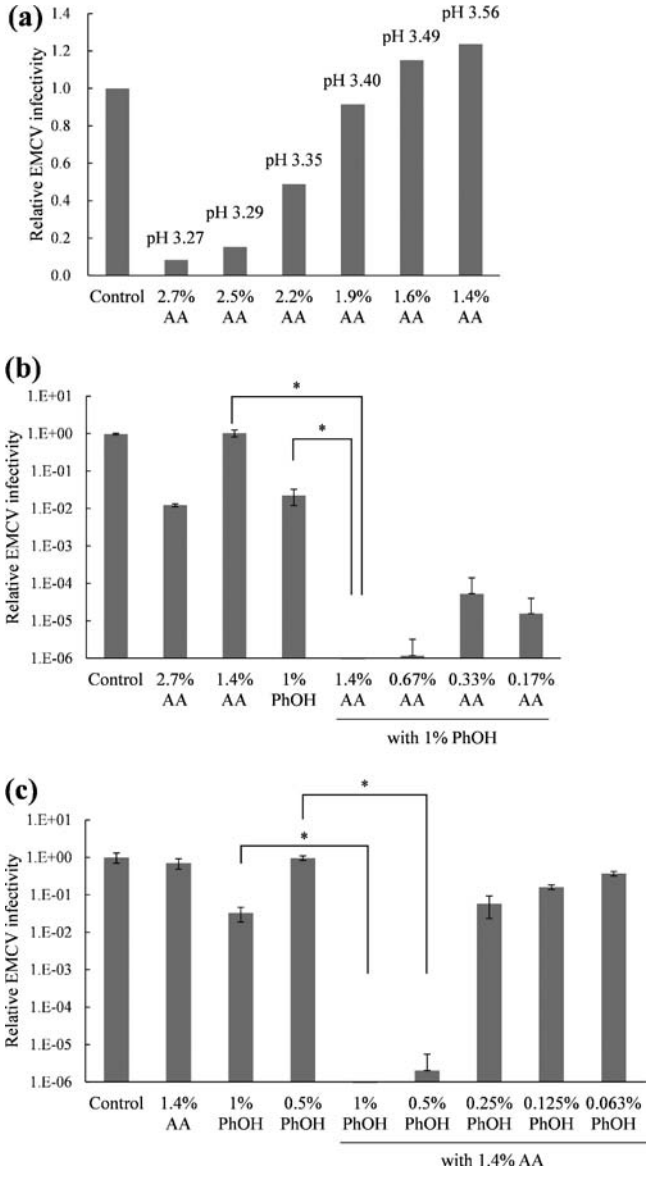

Figure 5. Synergistic effect of acetic acid and low-concentration phenol (PhOH) on EMCV inactivation: (a) EMCV inactivation assay using acetic acid (AA). Serially diluted AA was investigated using the viral inactivation assay and quantitative real-time PCR (b and c) EMCV inactivation assay using the combination of AA and PhOH. 2-fold serial dilution of AA (b) and PhOH (c) was investigated using the viral inactivation assay and quantitative real-time PCR. The pH value and the concentration indicate the conditions in the reaction mixtures of EMCV and the chemicals. *, $P < 0.05$ by Student's *t*-test.

methylguaiacol (35.2 mg/mL), and 4-ethylguaiacol (18.7 mg/mL) (Table 2). The finding of the EMCV inactivation assay using reconstructed Fr. 5 (Figure 3a, All) indicated that this fraction decreased EMCV infectivity to less than one-tenth of that with the control, which contains no BPA components. Therefore, the components responsible for the antiviral activity of Fr. 5 were analyzed by individually subtracting one of the 12 components from the reconstructed solution. As shown in Figure 3a, the EMCV-inactivating ability of reconstructed Fr. 5 disappeared when compound #5, 1% phenol, was removed from the reconstructed solution; however, subtraction of other compounds did not alter the EMCV-inactivating ability of the fraction. Moreover, compound #5 was the only chemical that exhibited high disinfectant activity against EMCV (Figure 3b). These results demonstrate that in Fr. 5, phenol is responsible for inactivation. In fact, phenol is a known germicide that kills bacteria, fungi, and viruses, and it has been demonstrated that 5% phenol exhibits broad-spectrum germicidal activity against viruses and completely inactivates not only lipid viruses (e.g., influenza A virus, vaccinia virus, and herpes simplex virus type 1) but also nonlipid viruses (e.g., EMCV, poliovirus, coxsackie B1 virus, and adenovirus type 2).[14] However, the virus-inactivating ability of BPA cannot be explained only by its phenol content because the concentration of phenol in BPA is less than (0.12% in BPA and 0.08% in dBPA) the minimum percentage (IC_{50} = 0.9%) that exhibits the germicidal activity against EMCV (Figure 4).

Synergistic Effect of Phenol and Acetic Acid on EMCV Infectivity. The germicidal activity of BPA cannot be explained by its phenol content because the phenol content is low; therefore, the potential synergistic effect of phenol and acetic acid, a major component of BPA was examined. As shown in Figure 5a, concentrations of acetic acid higher than 1.9% (pH 3.4) resulted in increased germicidal activity against EMCV. The inhibitory effects increased as the concentration of acetic acid increased, and 2.7% acetic acid decreased EMCV infectivity to one-tenth of that without acetic acid.

Phenol at a concentration of 1% also significantly decreased EMCV infectivity to one-fiftieth of the control (Figure 5b). Interestingly, although 1.4% acetic acid alone hardly affected the EMCV infectivity (Figure 5a and b), the combination of 1.4% acetic acid and 1% phenol completely abolished EMCV infectivity. This strong and drastic synergistic inhibition was observed even at lower acetic acid concentrations (0.17%−0.67%; Figure 5b). Despite the fact that at least 5% phenol is required to inactivate EMCV as well as other picornaviruses,[14] an even lower concentration of phenol was able to accomplish EMCV inactivation in combination with acetic acid in this assay. In an EMCV inactivation assay using 2-fold serial dilutions of 1% phenol, a combination of 0.5% phenol and 1.4% acetic acid produced a synergistic decrease in EMCV infectivity, even though the EMCV-inactivating ability was not observed when each chemical at the same concentration was used independently in the assay (Figure 5c). Furthermore, although a drastic synergistic effect was not observed at phenol concentrations <0.25%, the combination of 1.4% acetic acid with 0.25%, 0.125% and 0.625% phenol decreased EMCV infectivity to 5.8%, 16%, and 37%, respectively. Taken together, these data demonstrate that acetic acid augments the EMCV-inactivating ability of phenol, probably by changing the pH condition.

Some questions arise regarding the mechanism of the synergistic effect of acetic acid and phenol. The activity of low concentration (1%−2%) phenol as a germicide against vaccinia virus and herpes simplex type 1 virus is thought to arise from the inhibition and leakage of viral enzymes.[14,15] By contrast, picornaviruses do not carry any enzyme in their viral capsids, and therefore, viral genomic RNA must be translated in the beginning because it cannot be copied by any cellular RNA polymerase.[16] This means that viral RNA and/or capsid proteins should be affected by acetic acid and phenol in the assay. Moreover, it has been reported that (a) the icosahedral capsid of mengovirus, which belongs to the same *Cardiovirus* genus as EMCV, dissociates into pentamers at pH 6.2; (b) this dissociation proceeds with the release of intact viral genomes from virions and leads to the inactivation of mengovirus; and

(c) in case of EMCV, it cannot enter its target cells in acidic conditions; however, changing the pH from acidic to neutral restores EMCV infectivity.[17,18] Thus, one possibility is that the low pH caused by acetic acid, induces conformational changes of the EMCV capsid and makes it vulnerable to attack by phenol, resulting in the release of viral genome RNA from virion and the subsequent loss of viral infectivity.

On the basis of these results, BPA has a good, albeit not optimal, composition of acetic acid and phenol for inactivating EMCV. It would be amazing if the byproduct from sustainable materials can be used to inactivate virus. Because PA is thought to have lower environmental risk than chemical germicides, BPA can also be dispersed in the environment to kill other picornaviruses such as FMDV, which is one of the most devastating viruses affecting cloven-hoofed livestock, including cattle, sheep, goats, and pigs. FMDV is very sensitive to pH; hence, the acidic pH of BPA can interfere with FMDV infectivity, and the additional synergistic effect of phenol could strengthen the FMDV-inactivating activity of BPA. BPA is a potential germicide and may be useful for inactivating zoonotic viruses (e.g., avian influenza virus) as well as for preventing epidemics among livestock and humans.

Conclusions. PA, also called wood vinegar, is a byproduct of charcoal production. We demonstrated the germicidal activity of BPA against EMCV, and reconstruction of the active fraction using synthetic chemicals proved that phenol was the principal antiviral compound. Acetic acid, a major organic acid in BPA, significantly augmented the phenol's inactivating activity. Thus, the acidic medium containing phenol and acetic acid is a good combination for inactivating EMCV, and screening of the disinfectant activity of PA against a wide range of pathogenic viruses would lead to an extensive and safe application of PA in agricultural and human environments.

AUTHOR INFORMATION

Corresponding Author

*Tel: +81-774-38-3640. Fax: +81-774-38-3681. E-mail: twatanab@rish.kyoto-u.ac.jp.

Present Addresses

[⊥]Division of Molecular Immunology, Medical Mycology Research Center, Chiba University, Chiba 260-8673, Japan.
[#]The Aroma Library, Iidabashi, Tokyo 101-0072, Japan.

Author Contributions

[∥]These authors contributed equally to this work.

Funding

This work was supported by a grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Dr. Masahide Funabiki for experimental assistance.

ABBREVIATIONS USED

PA, pyroligneous acid; EMCV, encephalomyocarditis virus; BPA, bamboo pyroligneous acid; dBPA, distilled bamboo pyroligneous acid; GC-MS, gas chromatography–mass spectrometry; PCR, polymerase chain reaction

REFERENCES

(1) Cai, K.; Jiang, S.; Ren, C.; He, Y. Significant damage-rescuing effects of wood vinegar extract in living *Caenorhabditis elegans* under oxidative stress. *J. Sci. Food. Agric.* **2012**, *92*, 29–36.

(2) Hwang, Y. H.; Matsushita, Y. I.; Sugamoto, K.; Matsui, T. Antimicrobial effect of the wood vinegar from *Cryptomeria japonica* sapwood on plant pathogenic microorganisms. *J. Microbiol. Biotechnol.* **2005**, *15*, 1106–1109.

(3) Yatagai, M.; Unrinin, G.; Ohira, T. By-products of wood carbonization. IV. Components of wood vinegars. *Mokuzai. Gakkaishi.* **1988**, *34*, 184–188.

(4) Mohan, D.; Pittman, C. U., Jr.; Steele, P. H. Pyrolysis of wood/biomass for bio-oil: A critical review. Energy. *Fuels* **2006**, *20*, 848–889.

(5) Holley, R. A.; Patel, D. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food Microbiol.* **2005**, *22*, 273–292.

(6) Vitt, S. M.; Himelbloom, B. H.; Crapo, C. A. Inhibition of *Listeria innocua* and *L. monocytogenes* in a laboratory medium and cold-smoked salmon containing liquid smoke. *J. Food Saf.* **2001**, *21*, 111–125.

(7) Akakabe, Y.; Tamura, Y.; Iwamoto, S.; Takabayashi, M.; Nyuugaku, T. Volatile organic compounds with characteristic odor in bamboo vinegar. *Biosci. Biotechnol. Biochem.* **2006**, *70*, 2797–2799.

(8) Mun, S. P.; Ku, C. S. Pyrolysis GC-MS analysis of tars formed during the aging of wood and bamboo crude vinegars. *J. Wood. Sci.* **2010**, *56*, 47–52.

(9) Mu, J.; Uehara, T.; Furuno, T. Effect of bamboo vinegar on regulation of germination and radicle growth of seed plants. *J. Wood. Sci.* **2003**, *49*, 262–270.

(10) Mu, J.; Uehara, T.; Furuno, T. Effect of bamboo vinegar on regulation of germination and radicle growth of seed plants II: Composition of moso bamboo vinegar at different collection temperature and its effects. *J. Wood. Sci.* **2004**, *50*, 470–476.

(11) Miyamoto, Y.; Takeuchi, T.; Taniguchi, K. Inactivation of tobacco mosaic virus by "Mokusaku-eki. *Nihon Shokubutsu Byorigaku Kaihou* **1965**, *27*, 261.

(12) Lin, F. H.; Chueh, L. L.; Lee, Y. H.; Ho, I. The effects of montmorillonite and bamboo vinegar on porcine reproductive and respiratory syndrome virus. *Curr. Nanosci.* **2011**, *7*, 839–844.

(13) Yoneyama, M.; Kikuchi, M.; Natsukawa, T.; Shinobu, N.; Imaizumi, T.; Miyagishi, M.; Taira, K.; Akira, S.; Fujita, T. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat. Immunol.* **2004**, *5*, 730–737.

(14) Klein, M.; Deforest, A. In *Principles of Viral Inactivation*, 3rd ed.; Block, S. S., Ed.; Disinfection, Sterillization, and Preservation; Lea & Febiger: Philadelphia, PA, 1983; pp 422–434.

(15) Prindle, R. F. In *Phenolic Compounds*, 3rd ed.; Block, S. S., Ed.; Disinfection, Sterillization, and Preservation; Lea & Febiger: Philadelphia, PA, 1983; pp 197–224.

(16) Racaniello, V. R. In *Picornaviridae: The Viruses and Their Replication*, 5th ed.; Knipe, D. M., Howley, P. M., Eds.; Lippincott Williams & Wilkins: Philadelphia, PA, 2006; Fields Virology, Vol. *1*, pp 795–838.

(17) Madshus, I. H.; Olsnes, S.; Sandvig, K. Different pH requirements for entry of the two picornaviruses, human rhinovirus 2 and murine encephalomyocarditis virus. *Virology* **1984**, *139*, 346–357.

(18) Mak, T. W.; O'Callaghan, D. J.; Colter, J. S. Studies of the pH inactivation of three variants of Mengo encephalomyelitis virus. *Virology* **1970**, *40*, 565–571.