JOURNAL OF NATURAL PRODUCTS

pubs.acs.org/jnp

Article

Synthesis of Deoxyradicinin, an Inhibitor of *Xylella fastidiosa* and *Liberibacter crescens*, a Culturable Surrogate for *Candidatus* Liberibacter asiaticus

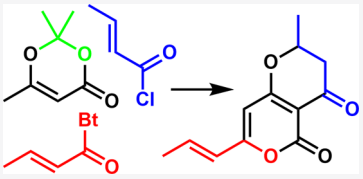
Connor A. Brandenburg, Claudia A. Castro, Alex A. Blacutt, Elizabeth A. Costa, Kyler C. Brinton, Diana W. Corral, Christopher L. Drozd, M. Caroline Roper, Philippe E. Rolshausen, Katherine N. Maloney, and Jonathan W. Lockner*

Cite This: *J. Nat. Prod.* 2020, 83, 1810−1816

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Pierce's disease of grapevine and citrus huanglongbing are caused by the bacterial pathogens *Xylella fastidiosa* and *Candidatus* Liberibacter asiaticus (CLas), respectively. Both pathogens reside within the plant vascular system, occluding water and nutrient transport, leading to a decrease in productivity and fruit marketability and ultimately death of their hosts. Field observations of apparently healthy plants in disease-affected vineyards and groves led to the hypothesis that natural products from endophytes may inhibit these bacterial pathogens. Previously, we showed that the natural product radicinin from *Cochliobolus* sp. inhibits *X. fastidiosa*. Herein we describe a chemical synthesis of deoxyradicinin and establish it as an inhibitor of both *X. fastidiosa* and *Liberibacter crescens*, a culturable surrogate for CLas. The key to this three-step route is a zinc-mediated enolate *C*-acylation, which allows for direct introduction of the propenyl side chain without extraneous redox manipulations.



Pierce's disease of grapevine and citrus huanglongbing (also known as citrus greening) are devastating plant diseases threatening important US agricultural industries. Both are caused by Gram-negative bacterial pathogens, spread by way of insect vectors. Pierce's disease, known in California since the 1800s, is caused by the bacterium *Xylella fastidiosa*, which inhabits the xylem of the plant.[1−3] As bacteria levels increase, the xylem becomes blocked and the flow of water and nutrients from the roots to the leaves is impeded, resulting in discolored and dried leaves, shriveled fruit, and stunted vine growth, ultimately leading to vine death.[4−6] *X. fastidiosa* is transmitted by xylem-feeding insects commonly known as leafhoppers. Native leafhoppers are limited in their flight range and transmission efficiency, and until recently, Pierce's disease outbreaks remained mostly isolated to small areas at a time. However, in 1989, an especially effective insect vector, the glassy-winged sharpshooter, was introduced to Southern California. The glassy-winged sharpshooter has infested most of Southern California, including the Malibu, Temecula Valley, and San Diego wine regions, and has made its way north into the Central Valley, posing an imminent threat to the California wine, raisin, and table grape industries.

Huanglongbing is caused by the unculturable bacterium *Candidatus* Liberibacter asiaticus (CLas).[7−9] Unlike *X. fastidiosa*, CLas colonizes the phloem (food transport tissue) of citrus trees. Huanglongbing-infected trees experience root loss, dieback, starch buildup, and mottled yellow leaves.[10,11] Affected fruit are often green, stunted, and bitter and may drop prematurely.[12,13] Huanglongbing can drastically impact the yield productivity of a tree and can ultimately prove fatal. CLas was introduced to Florida in 2005, where it was able to spread rapidly by way of its vector, the Asian citrus psyllid (in Florida since 1998), infecting virtually all Florida citrus trees and reducing production by nearly 75%.[14,15] CLas and Asian citrus psyllids have been making their way west. The Asian citrus psyllid was first detected in Southern California in 2008, and the first trees tested huanglongbing positive in 2012. The number of infected trees has been steadily increasing, and there are now over 1700 trees that have tested positive for huanglongbing.[16] Notably, these huanglongbing finds have been in backyard citrus trees, and huanglongbing has not yet been found in commercial citrus groves.

There is no known cure for either Pierce's disease or huanglongbing that targets the bacterium itself. A variety of approaches are being evaluated to control infection, including insecticide treatments to reduce insect vector populations, developing disease-resistant vines and trees through either breeding or genetic engineering, targeting the bacteria with bacteriophages or other bacteria, or removing infected plants.[17−19] Several small-molecule inhibitors of *L. crescens* (a culturable surrogate for CLas) have been identified. Tolfenamic acid inhibits a transcriptional accessory protein in the

Received: December 9, 2019
Published: June 8, 2020

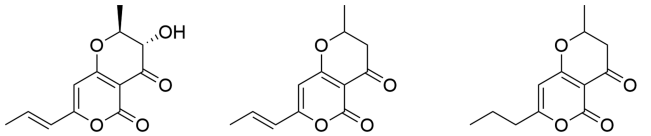
https://dx.doi.org/10.1021/acs.jnatprod.9b01207
J. Nat. Prod. 2020, 83, 1810−1816

bacterium.[20] Through structure-based drug design, polycyclic compounds (with IC_{50} values as low as 2.5 μM) were found to inhibit the protein translocase ATPase subunit SecA.[21] In a drug repurposing approach, several tetracyclines (at 0.8−1.9 μM), cefotaxime (at 0.7 μM), and penicillin V (at 0.1 μM) were found to provide nearly complete inhibition of *L. crescens*,[22] and recently streptomycin and oxytetracycline have been approved for spraying on Florida citrus groves under Section 18 emergency registration, although a recent study indicates that oxytetracycline foliar sprays are ineffective at controlling huanglongbing.[17,18,23,24]

An interesting phenomenon observed in vineyards and citrus groves infected with Pierce's disease and huanglongbing, respectively, suggested another possible avenue for fighting these diseases. Occasionally, apparently healthy plants can be found among the sick ones in areas of high disease pressure. These "disease-escaped vines" (in the case of Pierce's disease)[25,26] or "survivor trees" (in the case of huanglongbing)[27,28] show no or significantly reduced disease symptoms and slower progression of disease relative to their neighbors. Because both grapevines and citrus trees are clonally propagated, the mechanisms behind these disease-escaped/survivor phenotypes are likely not attributed to resistance/tolerance encoded in the plant's genome, leading us to explore plant endophytes—and their natural products—as a potential source for suppressors of *X. fastidiosa* and CLas.[26,29−31]

We previously reported the isolation of a *Cochliobolus* sp. from disease-escaped Chardonnay grapevines in Temecula and identified its major metabolite radicinin (**1**, Chart 1) as an inhibitor of *X. fastidiosa* in vitro.[26] To the best of our knowledge, **1** has not yet been examined against CLas or its culturable surrogate, *L. crescens*.

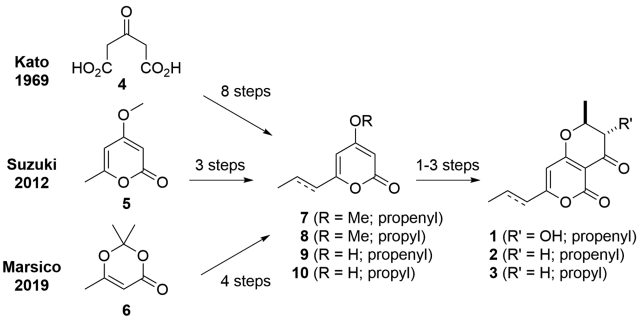
Chart 1. Structures of Radicinin (1), Deoxyradicinin (2), and Dihydrodeoxyradicinin (3)



radicinin (**1**) deoxyradicinin (**2**) dihydrodeoxyradicinin (**3**)

In the search for antibacterials for use in agricultural settings, synthetic approaches often complement isolation from natural sources. The first chemical synthesis of **1** was reported in 1969. Beginning with 3-oxoglutaric acid (**4**), racemic **1** was obtained in 11 steps (Scheme 1).[32] Recently, a synthesis of deoxyradicinin (**2**; biogenetic precursor to **1**) was disclosed, in which the target compound was obtained in five steps from commercially available **5**.[33] A caveat of this approach is the relatively high cost ($93.50 per gram) of this starting material. Moreover, these syntheses employ expensive and/or toxic [e.g., SeO_2, $Pb(OAc)_4$] reagents. Even the most recently reported synthesis of **2** (which came to light while the present article was in preparation) suffers from nonideal economies of synthesis, including recourse to an extraneous redox manipulation.[34] In order for an antibacterial agent to achieve widespread use in agricultural settings such as vineyards or citrus groves, an economical synthesis is needed. One aim of our work was to develop just such a synthesis of **1** and **2** (Scheme 1).

Scheme 1. Summary of Reported Syntheses of Radicinin (1), Deoxyradicinin (2), and Dihydrodeoxyradicinin (3)[a]



[a]Our approach permits access to a common intermediate in two steps and obtention of **2** or **3** in one additional step.

Given the agricultural threats posed by *X. fastidiosa* and CLas and the heavy reliance on insecticides to manage the lethal diseases they cause, antibacterial tactics are desperately needed. An ideal inhibitor of each bacterium (or both) would be cost-effective to produce, nontoxic to both flora and fauna, and easy to administer to infected plants. Secondary metabolites derived from endophytic fungi hold great appeal, because they are naturally endowed with many of these properties. Here, we further promote the radicinin family of natural products as useful antibacterials by demonstrating the antibacterial potencies of both natural **1** and synthetic **2**, against *X. fastidiosa* and *L. crescens* (a culturable surrogate for CLas).

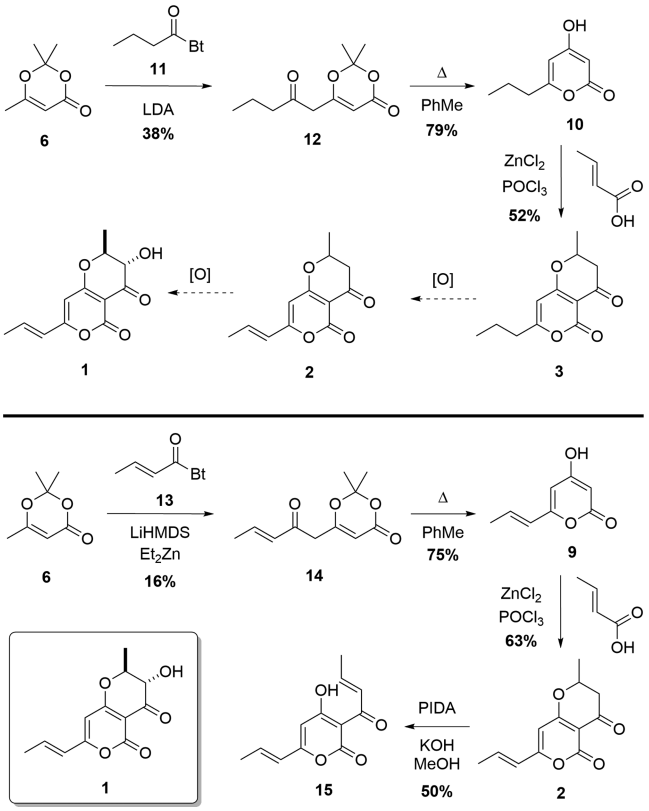
RESULTS AND DISCUSSION

Synthesis of Dihydrodeoxyradicinin (3), Deoxyradicinin (2), and Related Compounds. In 2001, Bach disclosed a means for securing 2-pyrones in three steps from dioxinone **6**.[35] Very recently, this strategy was followed by Marsico et al. for a synthesis of **2**.[36] However, this approach requires the preparation of a trimethylsilyl-protected enol ether, followed by Mukaiyama aldol addition and subsequent alcohol oxidation using Dess-Martin periodinane. To minimize step count, we preferred to avoid extraneous redox manipulations.[37] Furthermore, at the outset, our synthetic plan emphasized dihydrodeoxyradicinin (**3**) as the initial target (Scheme 2). We reasoned that late-stage oxidation of the propyl side chain (i.e., conversion of **3** to **2**) would permit us easier passage through the earlier stage of the synthesis. Given these considerations, we were drawn to direct enolate *C*-acylation tactics, and Katritzky's *N*-acyl benzotriazole tactic stood out among the array of options.[37−40] Among the virtues of *N*-acylbenzotriazoles is their ease of preparation, bench stability, and even commercial availability.[41]

Our three-step synthesis of dihydrodeoxyradicinin (**3**) commenced with *C*-acylation of the lithium enolate of dioxinone **6** with *N*-acylbenzotriazole **11** (derived from butyric acid) (Scheme 2). After column chromatography, the keto-dioxinone **12** was refluxed in anhydrous toluene to elicit an electrocyclization cascade, ultimately affording pyrone **10**. This intermediate precipitated out of the toluene solution, which provided for convenient isolation via filtration. Pyrone **10** was then treated with crotonic acid in the presence of $ZnCl_2$ and $POCl_3$[42] to afford the desired annulation product **3**. Although this sequence represents the most concise approach to **3**, we

https://dx.doi.org/10.1021/acs.jnatprod.9b01207
J. Nat. Prod. 2020, 83, 1810−1816

Scheme 2. Details for Our Syntheses of 3, 2, and 15[a]



[a]Bt = benzotriazol-1-yl; LDA = lithium diisopropylamide; LiHMDS = lithium hexamethyldisilazide; PIDA = phenyliodine(III) diacetate.

were unsuccessful in oxidizing **3** to **2** via the limited screening effort undertaken. Thus, we opted to pursue a three-step sequence for **2** that paralleled the synthesis of **3**.

Initially, we encountered a pitfall in our first step toward deoxyradicinin (**2**). Despite prior success in obtaining keto-dioxinone **12** en route to **3**, we were unsuccessful in isolating desired keto-dioxinone **14** using the original protocol for *C*-acylation. We reckoned that competing processes preclude the formation of **14** from **6** and **13**. The *N*-acylbenzotriazole **13** (derived from crotonic acid) bears acidic hydrogens, and it presents a further liability as a relatively unhindered α,β-unsaturated carbonyl moiety. Meanwhile, the lithium enolate of **6** is sufficiently basic to elicit undesired proton exchange with **13**. Making matters worse, the desired product **14** also bears acidic α-hydrogens, further stifling the success of this transformation. Fortunately for us, this is a problem that has been encountered in other contexts, and we were delighted to identify a solution that permitted us to proceed with our planned synthesis of **2**. In 1989, Morita introduced the notion of using an organozinc aid in alkylation and acylation of lithium enolates, demonstrating that "the presence of dimethylzinc in the reaction of lithium enolates and electrophiles effectively suppresses undesired α-proton exchange reaction and enhances the efficiency of enolate alkylation and acylation."[43] Since then, others have turned to alkylzincates (or zinc enolates) for *C*-acylations that were impossible with lithium enolates.[38,39,44,45] In our case, an alkylzincate (or zinc enolate) species derived from **6** is sufficiently less basic than the lithium enolate of **6**, permitting the formation and isolation of desired keto-dioxinone **14**. Gratifyingly, this turned out to be the case, and we were able to complete our three-step synthesis of deoxyradicinin (**2**) in a manner akin to that of **3**.

Having secured a three-step synthetic route from **6** to **2**, we next evaluated a few tactics for α-hydroxylation (i.e., **2** → **1**). In the pioneering 1969 synthesis, **2** was converted to **1** using superstoichiometric $Pb(OAc)_4$ followed by acetate hydrolysis. Preferring to avoid the use of this toxic reagent, we screened several iodine-based oxidation protocols.[46−49] We wish to make mention of only the salient observations from that effort. In all instances, unchanged **2** was recovered to some extent. Exposure of **2** to phenyliodine(III) diacetate (PIDA) in methanolic KOH afforded side-product **15**, a consequence of ring-opening of **2** (Scheme 2). Exposure of **2** to PIDA in refluxing $TFA/H_2O/CH_3CN$ afforded ca. 25% conversion of **2** to 3-*epi*-radicinin.

To complement our synthetic efforts, we evaluated conditions for chiral supercritical fluid chromatographic separation of the enantiomers of our synthetic racemic **3** as well as our synthetic racemic **2**. In lieu of synthetic radicinin (**1**), we made use of natural radicinin ((−)-**1**) isolated from *Alternaria radicina* (vide infra). With these compounds in hand, we were able to perform antibacterial assays.

Bioassay against *X. fastidiosa* and *L. crescens*. Compound **1** is known to inhibit *X. fastidiosa* in a dose-dependent fashion,[26] and we tested it, deoxyradicinin (**2**), and dihydrodeoxyradicinin (**3**) against *X. fastidiosa* and *L. crescens*. Having observed high potency of racemic **2** against *L. crescens* (vide infra), we performed chiral supercritical fluid chromatography (SFC) to obtain enantioenriched (−)-**2** and (+)-**2** for follow-up evaluation.

Against *X. fastidiosa*, **1** and **2** exhibit a comparable dose−response relationship (Figure S1). While **2** (racemic, synthetic in origin) is more potent than **1** (enantiopure, isolated from *A. radicina*) at lower doses, **1** provided complete inhibition (diameter = 10 cm) at the highest dose tested (2.12 μmol). Notably, **3** shows activity only at doses greater than 0.53 μmol, and even at the highest dose tested (2.12 μmol), it affords only a modest (diameter = 3.4 cm) level of inhibition. Thus, it appears that the propenyl side chain (serving as a conjugate acceptor) is vital for maintaining potency.

Against *L. crescens*, **1** and **2** again exhibit a comparable dose−response relationship, with both showing greater potency against *L. crescens* than against *X. fastidiosa* (Figure S2). Compared to **1** and **2**, compound **3** exhibited attenuated potency.

While **1** and its analogues have previously been reported to possess antibacterial activity against *X. fastidiosa*,[26] the capacity of **1**−**3** to inhibit *L. crescens* has not been detailed until now. As a note, the sample of **1** tested here was (−)-radicinin isolated from *Alternaria radicina*, whereas compounds **2** and **3** were synthesized as racemates. Racemic **2** as well as enantioenriched (−)-**2** and (+)-**2** were found to potently inhibit *L. crescens* (Figure S1).

We previously demonstrated the capacity of **1** and derivatives with the conjugate acceptor intact—but not derivatives lacking the conjugate acceptor—to deactivate *X. fastidiosa* proteases, consistent with enzymatic nucleophilic addition to **1**.[26] Thus, we expected **2** to inhibit *X. fastidiosa*. Our bioassay data confirm this in *X. fastidiosa* and expose a similar vulnerability in *L. crescens* (Figure S1). Comparable degrees of inhibition of *L. crescens* by enantioenriched samples of (−)- and (+)-deoxyradicinin[50] suggest that enzyme

https://dx.doi.org/10.1021/acs.jnatprod.9b01207
J. Nat. Prod. 2020, 83, 1810−1816

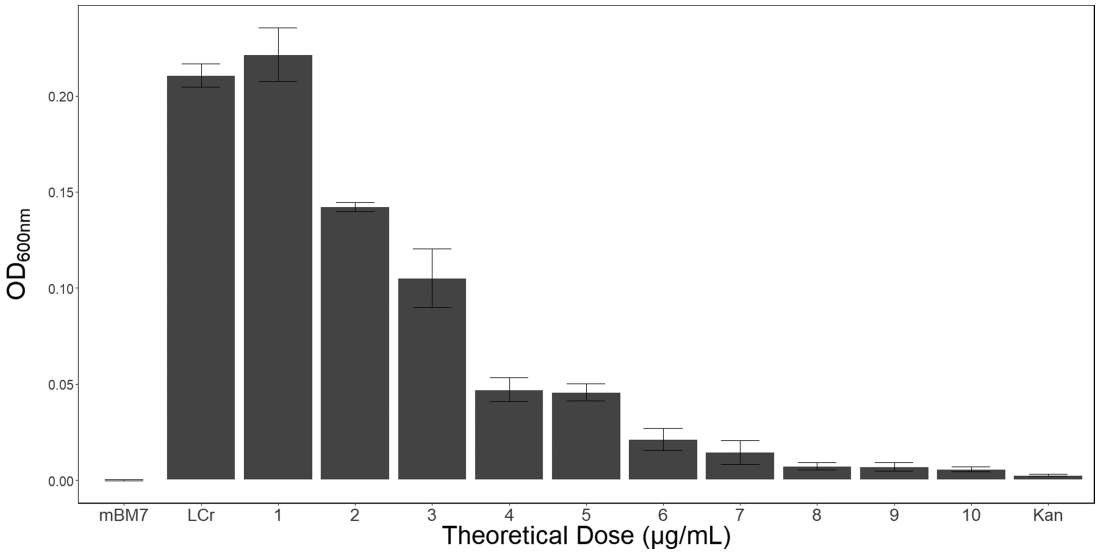


Figure 1. Minimum inhibitory concentration (MIC) for deoxyradicinin (**2**) against *L. crescens*. Theoretical dose refers to the applied concentration of **2**. Due to the poor aqueous solubility of **2**, the actual concentration was significantly lower (see text and Figure S4). Kan, 1.5 μg/mL kanamycin control; LCr, untreated *L. crescens*; mBM7, bBM7 + 1.0 mβc sterile growth media.

inactivation is not stereospecific,[51] a finding that bodes well from a developmental perspective.

Our previously published work on radicinin suggested an antibacterial mode of action involving enzyme (i.e., protease) inhibition via conjugate addition to an unhindered α,β-unsaturated carbonyl,[26] and our bioassay results with **2** and other analogues are consistent with this. Bioassay data on additional related compounds further suggests that the rigid bicyclic scaffold and bidentate 1,3-dicarbonyl are important features influencing antibacterial potency (Chart S1 and Figures S1 and S2).

Minimum Inhibitory Concentration of Deoxyradicinin. To further quantify the inhibitory effects of deoxyradicinin against *L. crescens*, we performed an inhibition assay in liquid bBM7 + 1.0 mβc medium and found the minimum inhibitory concentration (MIC)—the concentration at which no measurable bacterial growth occurs—at a theoretical dose of 8 μg/mL (Figure 1). However, the poor solubility of deoxyradicinin in aqueous media resulted in a discrepancy between the theoretical (applied) dose and the actual (measured) dose of soluble deoxyradicinin. As determined by LC-MS, an average of 44.8% of deoxyradicinin was solubilized in bBM7 + 1.0 mβc. Specifically, a dose of 8 μg/mL yields a concentration of solubilized deoxyradicinin of 3.5 ± 0.3 μg/mL (Figure S3). Thus, the MIC of soluble deoxyradicinin is 3.5 ± 0.3 μg/mL.

EXPERIMENTAL SECTION

General Experimental Procedures. All reactions were carried out in flame-dried glassware with magnetic stirring under an argon atmosphere, unless noted otherwise. ACS reagent grade or anhydrous dichloromethane (CH_2Cl_2), tetrahydrofuran (THF), and toluene were used without further purification. Compound **6** was obtained from Acros Organics (Morris Plains, NJ, USA). Compound **16** was obtained from Sigma-Aldrich (St. Louis, MO, USA). Compound **5** was prepared in two steps from **16** according to published methods.[52] Melting points were measured on an Electrothermal 1101D Mel-Temp digital melting point apparatus. Optical rotations were measured on an Atago Polax-2L polarimeter. Infrared spectra were recorded on a Thermo Scientific Nicolet iS10 FTIR spectrometer. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ (Cambridge Isotope Laboratories, Inc., Tewksbury, MA, USA) or DMSO-d_6 (Sigma-Aldrich) at 400 and 100 MHz, respectively, on a JEOL JNM-ECZ400S NMR spectrometer (JEOL, Ltd., Akishima, Tokyo, Japan). All spectra were referenced to residual solvent: 7.26 and 77.06 ppm for ^1H and ^{13}C NMR in $CDCl_3$; 2.50 and 39.53 ppm for ^1H and ^{13}C NMR in DMSO-d_6. Analytical and preparative thin layer chromatography (TLC) were performed on glass-backed silica gel plates. TLC plates were visualized by exposure to ultraviolet light and subsequently stained with *p*-anisaldehyde solution followed by heating. Flash column chromatography was performed on silica gel (0.040−0.063 mm, 230−400 mesh). Chiral supercritical fluid chromatography was performed on an SFC-PICLab HT with Open Bed fraction collection (PIC Solution SAS, Avignon, France). Dimethyl sulfoxide (DMSO), ethyl acetate (EtOAc), and methanol (MeOH) were used as solvents for filter disc preparation.

Preparation of 11.[53] To a solution of benzotriazole (28.6 g, 240.4 mmol) in CH_2Cl_2 (300 mL) was added $SOCl_2$ (7.1 g, 60.1 mmol) at 25 °C. After 30 min, butyric acid (5.3 g, 60.1 mmol) was added in one portion. After 2 h, the white precipitate was removed via filtration and rinsed with CH_2Cl_2 (2 × 50 mL). The filtrate was washed with 2 M NaOH (3 × 360 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel; 4:1 hexanes−EtOAc) to yield 1-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)butan-1-one (**11**) as an off-white solid (10.01 g, 88%): mp 59−60 °C (2-propanol); R_f = 0.6 (4:1 hexanes−EtOAc); ^1H NMR (400 MHz, $CDCl_3$) δ 8.30 (d, *J* = 8.2 Hz, 1 H), 8.11 (d, *J* = 8.2 Hz, 1 H), 7.65 (t, *J* = 7.8 Hz, 1 H), 7.50 (t, *J* = 7.8 Hz, 1 H), 3.40 (t, *J* = 7.3 Hz, 2 H), 1.94 (m, 2 H), 1.10 (t, *J* = 7.5 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 146.2, 130.4, 126.1, 120.2, 114.5, 100.0, 37.4, 18.0, 13.7. ^1H NMR and ^{13}C NMR data are consistent with those found in the literature.[54]

Preparation of 12.[37,38] To a solution of *N*,*N*-diisopropylamine (3.2 mL, 22.5 mmol) in anhydrous THF (60 mL) was added *n*-butyllithium (2.5 M in hexanes, 9.9 mL, 24.8 mmol) dropwise over 20 min at −78 °C. To this lithium diisopropylamide (LDA) solution was added a solution of **6** (2.4 mL, 17 mmol) in THF (60 mL) dropwise over 15 min at −78 °C. After 1.5 h, a solution of **11** (2.9 g, 15 mmol) in THF (60 mL) was added, and the reaction was warmed to room temperature overnight. The reaction was quenched with saturated aqueous NH_4Cl (6 mL) and concentrated in vacuo to afford a golden-brown syrup. Water (300 mL) was added to the syrup, and it was transferred to a separatory funnel for extraction with EtOAc (3 × 150 mL). The organic layers were combined, washed with saturated aqueous Na_2CO_3 (300 mL), dried over $MgSO_4$, filtered, and

https://dx.doi.org/10.1021/acs.jnatprod.9b01207
J. Nat. Prod. 2020, 83, 1810−1816

concentrated in vacuo. The residue was purified by flash column chromatography (silica gel; 1:1 hexanes−EtOAc) to yield 2,2-dimethyl-6-(2-oxopentyl)-4*H*-1,3-dioxin-4-one (**12**) as a colorless oil (1.21 g, 38%): R_f = 0.5 (1:1 hexanes−EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34 (s, 1 H), 3.31 (s, 2 H), 2.48 (t, J = 7.2 Hz, 2 H), 1.71 (s, 6 H), 1.67−1.61 (m, 2 H), 0.93 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.3, 164.7, 160.8, 107.2, 96.6, 47.1, 45.0, 25.0, 16.9, 13.5.

Preparation of 10.[32] A 0.1 M solution of **12** (1.11 g, 5.23 mmol) in anhydrous toluene (52.3 mL) was stirred under reflux for 1 h. The white precipitate was collected via filtration and washed with toluene (2 × 20 mL), yielding 4-hydroxy-6-propyl-2*H*-pyran-2-one (**10**) as an off-white solid (0.637 g, 79%): R_f = 0.3 (10:1 CH_2Cl_2−MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.33 (brs, 1 H), 6.01 (d, J = 2.1 Hz, 1 H), 5.59 (d, J = 2.1 Hz, 1 H), 2.45 (t, J = 7.5 Hz, 2 H), 1.70−1.61 (m, 2 H), 0.94 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 168.7, 167.2, 101.6, 89.9, 35.6, 20.2, 13.5; ^{1}H NMR and ^{13}C NMR data are consistent with those found in the literature.[55]

Preparation of Dihydrodeoxyradicinin (3).[42,56] To a solution of $POCl_3$ (2.4 g, 15.3 mmol) and $ZnCl_2$ (1.2 g, 8.8 mmol) were added crotonic acid (0.2 g, 2.19 mmol) and **10** (0.3 g, 2.2 mmol) at 85 °C with stirring for 4 h. The reaction was quenched with a small handful of ice and Na_2CO_3 (10% aq w/v %, 50 mL). The product was extracted with CH_2Cl_2 (2 × 100 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel; CH_2Cl_2 followed by 10:1 CH_2Cl_2−MeOH) to afford 2-methyl-7-propyl-2,3-dihydropyrano[4,3-*b*]pyran-4,5-dione (**3**) as a light brown solid (0.253 g, 52%): R_f = 0.4 (10:1 CH_2Cl_2−MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.88 (s, 1 H), 4.79−4.72 (m, 1 H), 2.71−2.58 (m, 2 H), 2.47 (t, J = 7.5 Hz, 2 H), 1.71 (sextet, J = 7.2 Hz, 2 H), 1.54 (d, J = 6.3 Hz, 3 H), 0.98 (t, J = 7.3 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.6, 176.0, 172.3, 158.1, 100.0, 99.1, 77.4, 43.8, 36.4, 20.4, 19.9, 13.5; ^{1}H NMR and ^{13}C NMR data are consistent with those found in the literature.[32,57] Analytical chiral SFC method screening identified a suitable means for separating (−)-**3** and (+)-**3**. Method conditions: Chiralpak AD-3, 4.6 × 100 mm, 3 μm; 10% isocratic in 5 min; 20 mM ammonium formate in MeOH; 3.5 mL/min; 160 bar; 25 °C; APCI (+); 1.0 μL injection. Preparative-scale separation was not done for **3**.

Preparation of 13.[53] To a solution of benzotriazole (28.6 g, 240.4 mmol) in CH_2Cl_2 (300 mL) was added $SOCl_2$ (7.1 g, 60.1 mmol) at 25 °C. After 30 min, crotonic acid (5.2 g, 60.1 mmol) was added in one portion. After 2 h, the white precipitate was removed via filtration and rinsed with CH_2Cl_2 (2 × 50 mL). The filtrate was washed with 2 M NaOH (3 × 360 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel; 4:1 hexanes−EtOAc) to yield (*E*)-1-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)but-2-en-1-one (**13**) as an off-white solid (9.96 g, 89%): mp 91−93 °C (2-propanol); R_f = 0.6 (4:1 hexanes−EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, J = 8.2 Hz, 1 H), 8.12 (d, J = 8.5 Hz, 1 H), 7.73−7.59 (m, 1 H), 7.57−7.43 (m, 3 H), 2.12 (d, J = 5.5 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.6, 150.0, 146.3, 131.4, 130.2, 126.2, 121.4, 120.1, 114.8, 19.9; ^{1}H NMR and ^{13}C NMR data are consistent with those found in the literature.[53]

Preparation of 14.[37,38] To anhydrous THF (84 mL) was added hexamethyldisilazane (8.8 mL, 42 mmol) at −78 °C. *n*-Butyllithium (16.8 mL, 42 mmol, 2.5 M in hexanes) was added dropwise over 10 min. After 20 min, a solution of **6** (4 mL, 30 mmol) in THF (12 mL) was added dropwise over 10 min. After 1 h, diethylzinc (42 mL, 42 mmol, 1.0 M in hexanes) was slowly added. After 20 min, the mixture was warmed to −20 °C. A solution of **13** (6.75 g, 36 mmol) in THF (18 mL) was added, and stirring was continued for 2 h. The reaction was quenched with 1 M HCl (240 mL), and the aqueous layer was acidified to pH 1−2 using 1 M HCl. The product was extracted with EtOAc (2 × 300 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel; 3:1 hexanes−EtOAc) to yield 2,2-dimethyl-6-[(3*E*)-2-oxopent-3-en-1-yl]-2,4-dihydro-1,3-dioxin-4-one (**14**) as a colorless oil (1.016 g, 16%): R_f = 0.5 (1:1 hexanes−EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92 (dq, J = 15.6, 6.9 Hz, 1 H), 6.16 (m, 1 H), 5.36 (s, 1 H), 3.44 (s, 2 H), 1.94 (m, 3 H), 1.69 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.5, 165.2, 161.0, 145.6, 130.9, 107.4, 96.8, 44.7, 25.1, 18.5; ^{1}H NMR and ^{13}C NMR data are consistent with those found in the literature.[35]

Preparation of 9.[32] A 0.1 M solution of **14** (0.228 g, 1.09 mmol) in anhydrous toluene (10.9 mL) was stirred under reflux for 10 min. The white precipitate was collected via filtration and washed with toluene (2 × 10 mL), yielding (*E*)-4-hydroxy-6-(prop-1-en-1-yl)-2*H*-pyran-2-one (**9**) as an off-white solid (0.124 g, 75%): mp 183−185 °C; R_f = 0.3 (10:1 CH_2Cl_2−MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.63 (s, 1 H), 6.46 (dq, J = 15.6, 6.9 Hz, 1 H), 6.15 (dd, J = 15.6, 1.4 Hz, 1 H), 5.98 (d, J = 1.8 Hz, 1 H), 5.22 (d, J = 1.8 Hz, 1 H), 1.82 (dd, J = 6.9, 1.4 Hz, 3 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.4, 163.1, 159.0, 133.8, 123.4, 99.8, 89.4, 18.1; ^{1}H NMR and ^{13}C NMR data are consistent with those found in the literature.[35,58]

Preparation of Deoxyradicinin (2).[42,56] To a solution of $POCl_3$ (0.4 mL, 4.4 mmol) and $ZnCl_2$ (0.3 g, 2.5 mmol) were added crotonic acid (0.05 g, 0.6 mmol) and **9** (0.097 g, 0.6 mmol) at 85 °C with stirring for 4 h. The reaction was quenched with a small handful of ice and Na_2CO_3 (10% aq w/v %, 15 mL). The product was extracted with CH_2Cl_2 (2 × 40 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel; CH_2Cl_2 followed by 10:1 CH_2Cl_2−MeOH) to afford (*E*)-2-methyl-7-(prop-1-en-1-yl)-2,3-dihydropyrano[4,3-*b*]pyran-4,5-dione (**2**) as a dark brown solid (0.091 g, 63%): R_f = 0.4 (10:1 CH_2Cl_2−MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99−6.87 (m, 1 H), 6.02 (dd, J = 15.4, 1.6 Hz, 1 H), 5.83 (s, 1 H), 4.80−4.69 (m, 1 H), 2.70−2.58 (m, 2 H), 1.95 (dd, J = 7.0, 1.6 Hz, 3 H), 1.53 (d, J = 6.4 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$)δ 187.5, 177.1, 164.5, 155.5, 141.2, 118.2, 99.9, 93.1, 76.7, 39.1, 20.9, 17.8; ^{1}H NMR and ^{13}C NMR data are consistent with those found in the literature.[32,59] Preparative chiral SFC furnished separate samples of enantioenriched (−)-**2** and (+)-**2**. Method conditions: Chiralpak AS-3, 4.6 × 100 mm, 3 μm; 20% isocratic in 2.0 min; 20 mM ammonium formate in MeOH; 3.5 mL/min; 160 bar; 25 °C; APCI (+); 3.0 μL injection.

Preparation of 15.[46] To a solution of KOH (0.056 g, 1.0 mmol) in MeOH (2 mL) at 0 °C was added **2** (0.022 g, 0.10 mmol) over 10 min. Phenyliodine(III) diacetate (0.064 g, 0.20 mmol) was added over 10 min, and the reaction allowed to warm to room temperature overnight. The reaction was quenched with 0.1 M HCl (0.2 mL). The product was extracted with CH_2Cl_2 (2 × 10 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (1:1 hexanes−acetone) to afford 3-((*E*)-but-2-enoyl)-4-hydroxy-6-((*E*)-prop-1-en-1-yl)-2*H*-pyran-2-one (**15**) as an off-white solid (11 mg, 50%): R_f = 0.4 (1:1 hexanes−acetone); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, J = 15.3 Hz, 1 H), 7.34−7.23 (m, 1 H), 6.92 (dq, J = 15.2, 7.0 Hz, 1 H), 6.03 (d, J = 15.5 Hz, 1 H), 5.88 (s, 1 H), 2.02 (d, J = 6.9 Hz, 3 H), 1.96 (d, J = 6.9 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.0, 183.2, 163.4, 160.8, 147.4, 140.2, 128.3, 122.9, 100.8, 99.6, 19.1, 18.9.

Isolation of (−)-Radicinin (1). (−)-Radicinin was obtained from the fermentation of *Alternaria radicina* (ATCC 96831) shaken at 190 rpm in potato dextrose broth for 24 days. ^{1}H NMR data are consistent with those found in the literature.[26,32,59] Analytical chiral SFC confirmed the enantiopurity of (−)-**1** (see Supporting Information). Method conditions: Chiralpak AD-3, 4.6 × 100 mm, 3 μm; 40% isocratic in 5 min; 20 mM ammonium formate in MeOH; 3.5 mL/min; 160 bar; 25 °C; APCI (+); 1.0 μL injection.

Antibacterial Assays. Compounds were evaluated using an *in vitro* assay of their ability to inhibit *X. fastidiosa* or *L. crescens* growth. Compounds were dissolved in DMSO, EtOAc, or MeOH and applied to sterile filter discs (Difco) to achieve desired doses. Culture techniques for each bacterium are described below. After incubation with compound-loaded filter discs at 28 °C for 5 to 7 days, the diameters of clear zones of inhibition were measured and recorded. Each assay was performed in triplicate, and the average diameter of inhibition reported. Compounds that afforded significant inhibition at

https://dx.doi.org/10.1021/acs.jnatprod.9b01207
J. Nat. Prod. 2020, 83, 1810−1816

higher doses were tested at lower doses to further characterize dose response.

***Xylella fastidiosa* Inhibition Assay.** Pierce's disease medium (PD3)[60] top agar (0.8% agar) was prepared, cooled to 60 °C, and amended 10% v/v with 6-day-old *X. fastidiosa* liquid culture (PD3, 28 °C, 180 rpm shaking), and the OD_{600} was adjusted to 0.1. This amended top agar was then dispensed to evenly coat previously poured PD3 agar plates. After 2 days of incubation at 28 °C, compound-loaded filter discs were placed in the center of the plates.

***Liberibacter crescens* Inhibition Assay.** bBM7 + 1.0 mβc top agar[61] (0.8% agar) was prepared, cooled to 60 °C, and amended 10% v/v with 4-day-old *L. crescens* liquid culture (mBM7, 28 °C, 180 rpm shaking). This amended top agar was then dispensed to evenly coat previously poured bBM7 + 1.0 mβc agar plates, and compound-loaded filter discs were placed in the center of the plates. See Jain et al.[62] for justification for the use of *L. crescens* as a culturable surrogate for CLas.

Minimum Inhibitory Concentration Assay for Deoxyradicinin against *L. crescens*. The minimum inhibitory concentration was calculated for deoxyradicinin in liquid bBM7 + 1.0 mβc as described previously.[31] In brief, deoxyradicinin was dissolved in methanol, filter sterilized, and added to a 96-well plate to give concentrations from 0.5 to 10 μg/mL. Methanol was evaporated off in a biosafety cabinet overnight. The following day, 150 μL of sterile bBM7 + 1.0 mβc was added to the wells, along with 50 μL of 4-day-old liquid culture ($OD_{600\ nm}$ ~0.27) of *L. crescens*. The plate was incubated at 28 °C at 150 rpm. Absorbance was read at 600 nm using an Infinite 200 Pro plate reader (Tecan Group Ltd., Switzerland) daily for 11 days. The lowest dose at which no growth was observed was recorded as the MIC. Each treatment had four technical replicates.

***In Vitro* Solubility Assay for Deoxyradicinin.** For quantification of solubilized deoxyradicinin, 96-well plates containing bBM7 + 1.0 mβc media were prepared as described above, but with no bacteria. Three concentrations were tested for solubility: 7.5, 8, and 8.5 μg/mL. Plates were incubated at 28 °C at 150 rpm for 48 h and 150 μL aliquots were analyzed via LC-MS. Prior to LC-MS analysis, protein was precipitated with methanol. After centrifugation at 16000*g* for 15 min at 4 °C, supernatant was analyzed by LC-MS. LC-MS was performed at the UC Riverside Metabolomics Core Facility on a Synapt G2-Si quadrupole time-of-flight mass spectrometer (Waters) coupled to an I-class UPLC system (Waters). Separations were carried out on a CSH phenyl-hexyl column (2.1 × 100 mm, 1.7 μM) (Waters). The mobile phases were (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. The flow rate was 250 μL/min, and the column was held at 40 °C. The injection volume was 1 μL. The gradient was as follows: 0 min, 1% B; 1 min, 1% B; 8 min, 40% B; 8.5 min, 100% B; 10 min, 100% B; 10.5 min, 1% B. The MS was operated in positive ion mode (50 to 1200 m/z) with a 100 ms scan time. Source and desolvation temperatures were 150 and 600 °C, respectively. Desolvation gas was set to 1100 L/h and cone gas to 150 L/h. All gases were nitrogen except the collision gas, which was argon. Capillary voltage was 1 kV. Samples were analyzed in random order. Leucine enkephalin was infused and used for mass correction. Data were analyzed using QuanLynx software (Waters).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.9b01207.

Supplemental figures, ^1H and ^{13}C NMR spectra, and chiral SFC chromatograms (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Jonathan W. Lockner − *Department of Chemistry, Point Loma Nazarene University, San Diego, California 92106, United States;* orcid.org/0000-0001-6148-0579; Email: jlockner@pointloma.edu

Authors

Connor A. Brandenburg − *Department of Chemistry, Point Loma Nazarene University, San Diego, California 92106, United States*

Claudia A. Castro − *Department of Microbiology and Plant Pathology, University of California, Riverside, California 92521, United States*

Alex A. Blacutt − *Department of Microbiology and Plant Pathology, University of California, Riverside, California 92521, United States*

Elizabeth A. Costa − *Pfizer Inc., La Jolla, California 92121, United States*

Kyler C. Brinton − *Department of Chemistry, Point Loma Nazarene University, San Diego, California 92106, United States*

Diana W. Corral − *Department of Chemistry, Point Loma Nazarene University, San Diego, California 92106, United States*

Christopher L. Drozd − *Department of Microbiology and Plant Pathology, University of California, Riverside, California 92521, United States*

M. Caroline Roper − *Department of Microbiology and Plant Pathology, University of California, Riverside, California 92521, United States*

Philippe E. Rolshausen − *Department of Botany and Plant Sciences, University of California, Riverside, California 92521, United States*

Katherine N. Maloney − *Department of Chemistry, Point Loma Nazarene University, San Diego, California 92106, United States;* orcid.org/0000-0003-3472-7593

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jnatprod.9b01207

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We gratefully acknowledge Point Loma Nazarene University (PLNU) for supporting this work through research grants from the Department of Chemistry and Research Associates. This research was also funded by the California Department of Food and Agriculture (CDFA) Pierce's Disease/Glassy-Winged Sharpshooter (PD/GWSS) Board (Grant #1687), and the United States Department of Agriculture (USDA Project No. CA-R-PPA-5139-CG). We also gratefully acknowledge Lia Lozano and Jennifer Cordoza of PLNU for providing (−)-radicinin (**1**) for bioassay and Dr. Chambers Hughes and Trevor Purdy of the Scripps Institution of Oceanography for assistance in acquiring mass spectrometry data.

■ REFERENCES

(1) Varela, L. G.; Smith, R. J.; Phillips, P. A. *Pierce's Disease*, Publication 21600; University of California Agricultural and Natural Resources: Oakland, CA, 2001.

(2) Wells, J. M.; Raju, B. C.; Hung, H.-Y.; Weisburg, W. G.; Mandelco-Paul, L.; Brenner, D. J. *Int. J. Syst. Bacteriol.* **1987**, *37*, 136−143.

(3) Hopkins, D. *Annu. Rev. Phytopathol.* **1989**, *27*, 271−290.

(4) Hopkins, D. L.; Purcell, A. H. *Plant Dis.* **2002**, *86*, 1056−1066.

(5) Thorne, E. T.; Stevenson, J. F.; Rost, T. L.; Labavitch, J. M.; Matthews, M. A. *Am. J. Enol. Vitic.* **2006**, *57*, 1−11.

(6) Choi, H.-K.; Iandolino, A.; da Silva, F. G.; Cook, D. R. *Mol. Plant-Microbe Interact.* **2013**, *26*, 643−657.

(7) Chen, J.; Deng, X.; Sun, X.; Jones, D.; Irey, M.; Civerolo, E. *Phytopathology* **2010**, *100*, 567−572.
(8) Kunta, M.; Zheng, Z.; Wu, F.; da Graca, J. V.; Park, J.-W.; Deng, X.; Chen, J. *Genome Announc.* **2017**, *5*, No. e00170-17.
(9) Zheng, Z.; Wu, F.; Kumagai, L. B.; Polek, M.; Deng, X.; Chen, J. *Phytopathology* **2017**, *107*, 662−668.
(10) Graça, J. V. da; da Graça, J. V.; Douhan, G. W.; Halbert, S. E.; Keremane, M. L.; Lee, R. F.; Vidalakis, G.; Zhao, H. *J. Integr. Plant Biol.* **2016**, *58*, 373−387.
(11) Bove, J. M.; Pereira de Barros, A. *J. Plant Pathol.* **2006**, *88*, 7−37.
(12) Bassanezi, R. B.; Montesino, L. H.; Stuchi, E. S. *Eur. J. Plant Pathol.* **2009**, *125*, 565−572.
(13) Dagulo, L.; Danyluk, M. D.; Spann, T. M.; Valim, M. F.; Goodrich-Schneider, R.; Sims, C.; Rouseff, R. *J. Food Sci.* **2010**, *75*, C199−C207.
(14) Folimonova, S. Y.; Robertson, C. J.; Garnsey, S. M.; Gowda, S.; Dawson, W. O. *Phytopathology* **2009**, *99*, 1346−1354.
(15) Gottwald, T. R.; Graham, J. H.; Irey, M. S.; McCollum, T. G.; Wood, B. W. *Crop Prot.* **2012**, *36*, 73−82.
(16) Karp, D. California Farmers and Scientists Race to Combat a Citrus Disease Infecting Trees. *Los Angeles Times*. **2019**, March 29.
(17) Blaustein, R. A.; Lorca, G. L.; Teplitski, M. *Phytopathology* **2018**, *108*, 424−435.
(18) McKenna, M. *Nature* **2019**, *567*, 302−303.
(19) Kyrkou, I.; Pusa, T.; Ellegaard-Jensen, L.; Sagot, M.-F.; Hansen, L. H. *Front. Microbiol.* **2018**, *9*, 2141.
(20) Gardner, C. L.; Pagliai, F. A.; Pan, L.; Bojilova, L.; Torino, M. I.; Lorca, G. L.; Gonzalez, C. F. *Front. Microbiol.* **2016**, *7*, 1630.
(21) Akula, N.; Trivedi, P.; Han, F. Q.; Wang, N. *Eur. J. Med. Chem.* **2012**, *54*, 919−924.
(22) Turpen, T. Discussion of Penicillin, Antimicrobial R&D and Bactericide Now Initiative, **2014**. https://citrusrdf.org/wp-content/uploads/2019/04/Antimicrobials.pdf.
(23) Belasque, J., Jr.; Yamamoto, P. T.; de Miranda, M. P.; Bassanezi, R. B.; Ayres, A. J.; Bové, J. M. *Citrus Res. & Technol.* **2010**, *31*, 53−64.
(24) Li, J.; Pang, Z.; Duan, S.; Lee, D.; Kolbasov, V. G.; Wang, N. *Phytopathology* **2019**, *109*, 2046−2054.
(25) Darjean-Jones, D. Chemical and Biological Strategies for the Management of Xylella Fastidiosa, Causal Agent of Pierce's Disease of Grapevine. Ph.D. Thesis, University of California, Davis, 2004.
(26) Aldrich, T. J.; Rolshausen, P. E.; Roper, M. C.; Reader, J. M.; Steinhaus, M. J.; Rapicavoli, J.; Vosburg, D. A.; Maloney, K. N. *Phytochemistry* **2015**, *116*, 130−137.
(27) Wang, N.; Gmitter, F. *Citrus Industry* **2014**, 16−17.
(28) Riera, N.; Handique, U.; Zhang, Y.; Dewdney, M. M.; Wang, N. *Front. Microbiol.* **2017**, *8*, 2415.
(29) Deyett, E.; Roper, M. C.; Ruegger, P.; Yang, J.-I.; Borneman, J.; Rolshausen, P. E. *Phytobiomes J.* **2017**, *1*, 138−149.
(30) Ginnan, N. A.; Dang, T.; Bodaghi, S.; Ruegger, P. M.; Peacock, B. B.; McCollum, G.; England, G.; Vidalakis, G.; Roper, C.; Rolshausen, P.; Borneman, J. *Phytobiomes J.* **2018**, *2*, 64−70.
(31) Blacutt, A.; Ginnan, N.; Dang, T.; Bodaghi, S.; Vidalakis, G.; Ruegger, P.; Peacock, B.; Viravathana, P.; Vieira, F. C.; Drozd, C.; Jablonska, B.; Borneman, J.; McCollum, G.; Cordoza, J.; Meloch, J.; Berry, V.; Salazar, L. L.; Maloney, K. N.; Rolshausen, P. E.; Roper, M. C. *Appl. Environ. Microbiol.* **2020**, *86*, No. e02883-19.
(32) Kato, K.; Hirata, Y.; Yamamura, S. *J. Chem. Soc. C* **1969**, *0*, 1997−2002.
(33) Suzuki, M.; Sakuno, E.; Ishihara, A.; Tamura, J.-I.; Nakajima, H. *Phytochemistry* **2012**, *75*, 14−20.
(34) Marsico, G.; Ciccone, M. S.; Masi, M.; Freda, F.; Cristofaro, M.; Evidente, A.; Superchi, S.; Scafato, P. *Molecules* **2019**, *24*, 3193.
(35) Bach, T.; Kirsch, S. *Synlett* **2001**, *2001*, 1974−1976.
(36) Newhouse, T.; Baran, P. S.; Hoffmann, R. W. *Chem. Soc. Rev.* **2009**, *38*, 3010−3021.
(37) Katritzky, A. R.; Wang, Z.; Wang, M.; Hall, C. D.; Suzuki, K. *J. Org. Chem.* **2005**, *70*, 4854−4856.
(38) Patel, B. H.; Mason, A. M.; Barrett, A. G. M. *Org. Lett.* **2011**, *13*, 5156−5159.
(39) McDonald, S. L.; Wang, Q. *Chem. Commun.* **2014**, *50*, 2535−2538.
(40) Reber, K. P.; Burdge, H. E. *J. Nat. Prod.* **2018**, *81*, 292−297.
(41) *N*-Acylbenzotriazoles are commercially available (e.g., see Table S1), but their preparation is trivial and details are provided in the Experimental Section of this paper.
(42) Zehnder, L. R.; Dahl, J. W.; Hsung, R. P. *Tetrahedron Lett.* **2000**, *41*, 1901−1905.
(43) Morita, Y.; Suzuki, M.; Noyori, R. *J. Org. Chem.* **1989**, *54*, 1785−1787.
(44) Arisawa, M.; Torisawa, Y.; Kawahara, M.; Yamanaka, M.; Nishida, A.; Nakagawa, M. *J. Org. Chem.* **1997**, *62*, 4327−4329.
(45) Hlavinka, M. L.; Hagadorn, J. R. *Organometallics* **2007**, *26*, 4105−4108.
(46) Moriarty, R. M.; Hu, H.; Gupta, S. C. *Tetrahedron Lett.* **1981**, *22*, 1283−1286.
(47) Moriarty, R. M.; Berglund, B. A.; Penmasta, R. *Tetrahedron Lett.* **1992**, *33*, 6065−6068.
(48) Liu, W.; Chen, C.; Zhou, P. *J. Org. Chem.* **2017**, *82*, 2219−2222.
(49) Yang, J.; Xie, D.; Zhou, H.; Chen, S.; Huo, C.; Li, Z. *Org. Chem. Front.* **2018**, *5*, 1325−1329.
(50) Chiral SFC separation of synthetic racemic deoxyradicinin (**2**) was performed by Pfizer Inc. See Supporting Information for details.
(51) Masi, M.; Meyer, S.; Clement, S.; Cimmino, A.; Cristofaro, M.; Evidente, A. *J. Nat. Prod.* **2017**, *80*, 1241−1247.
(52) Soldi, C.; Moro, A. V.; Pizzolatti, M. G.; Correia, C. R. D. *Eur. J. Org. Chem.* **2012**, *2012*, 3607−3616.
(53) Katritzky, A. R.; Zhang, Y.; Singh, S. K. *Synthesis* **2003**, 2795−2798.
(54) Katritzky, A. R.; Huang, T. B.; Voronkov, M. V.; Steel, P. J. *J. Org. Chem.* **2000**, *65*, 8069−8073.
(55) Hsung, R.; Zhang, X.; McLaughlin, M.; Muñoz, R.; Wang, J.; Swidorski, J. *Synthesis* **2007**, *2007*, 749−753.
(56) Shah, V.; Bose, J.; Shah, R. *J. Org. Chem.* **1960**, *25*, 677−678.
(57) Kato, K.; Hirata, Y.; Yamamura, S. *Chem. Commun. (London)* **1968**, *0*, 319.
(58) Suzuki, E.; Hamajima, R.; Inoue, S. *Synthesis* **1975**, *1975*, 192−194.
(59) Kato, K.; Hirata, Y.; Yamamura, S. *J. Chem. Soc. D* **1969**, *0*, 95.
(60) Davis, M. J.; French, W. J.; Schaad, N. W. *Curr. Microbiol.* **1981**, *6*, 309−314.
(61) Naranjo, E.; Merfa, M. V.; Ferreira, V.; Jain, M.; Davis, M. J.; Bahar, O.; Gabriel, D. W.; De La Fuente, L. *Sci. Rep.* **2019**, *9*, 5150.
(62) Jain, M.; Cai, L.; Fleites, L. A.; Munoz-Bodnar, A.; Davis, M. J.; Gabriel, D. W. *Phytopathology* **2019**, *109*, 1811−1819.

https://dx.doi.org/10.1021/acs.jnatprod.9b01207
J. Nat. Prod. 2020, 83, 1810−1816