JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Phomentrioloxin, a Fungal Phytotoxin with Potential Herbicidal Activity, and its Derivatives: A Structure−Activity Relationship Study

Alessio Cimmino,[†] Anna Andolfi,[†] Maria Chiara Zonno,[‡] Angela Boari,[‡] Ciro Troise,[†] Andrea Motta,[§] Maurizio Vurro,[‡] Gavin Ash,[∥] and Antonio Evidente*[,†]

[†]Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Complesso Universitario Monte S. Angelo, Via Cintia 4, 80126 Napoli, Italy

[‡]Istituto di Scienze delle Produzioni Alimentari, Consiglio Nazionale delle Ricerche, Via Amendola 122/O, 70125 Bari, Italy

[§]Istituto di Chimica Biomolecolare, Consiglio Nazionale delle Ricerche, Comprensorio Olivetti, Edificio 70, Via Campi Flegrei 34, 80078 Pozzuoli, Italy

[∥]Graham Centre for Agriculture Innovation (an Alliance between Charles Sturt University and NSW DPI), Booroma Street, Locked Bag 588, Wagga Wagga, New South Wales 2678, Australia

Supporting Information

ABSTRACT: Phomentrioloxin is a phytotoxic geranylcyclohexenetriol produced in liquid culture by *Phomopsis* sp. (teleomorph: *Diaporthe gulyae*), a potential mycoherbicide proposed for the control of the annual weed *Carthamus lanatus*. In this study, seven derivatives obtained by chemical modifications of the toxin were assayed for phytotoxic, antimicrobial, and zootoxic activities, and the structure−activity relationships were examined. Each compound was tested on nonhost weedy and agrarian plants, fungi, Gram+ and Gram− bacteria, and on brine shrimp larvae. The results provide insights into an investigation of the structural requirements for activity. The hydroxy groups at C-2 and C-4 appeared to be important features for the phytotoxicity, as well as an unchanged cyclohexentriol ring. A role seemed also to be played by the unsaturations of the geranyl side chain. These findings could be useful for understanding the mechanisms of action of new natural products, for identifying the active sites, and possibly in devising new herbicides of natural origin.

KEYWORDS: *Phomopsis sp., Diaporthe gulyae, weeds, Carthamus lanatus, phytotoxins, phomentrioloxin, SAR*

INTRODUCTION

Carthamus lanatus L. (common name saffron thistle) is a widespread and troublesome weed belonging to the Asteraceae family, causing severe crop and pasture losses in Australia, and a *Phomopsis* sp. strain was proposed for its biocontrol.[1,2] Recently, the teleomorph of the pathogen was classified as *Diaporthe gulyae*,[3] and from its culture filtrates a new phytotoxic geranylhydroquinone named phomentrioloxin (**1**, Figure 1) was isolated. It was characterized as (1*S*,2*S*,3*S*,4*S*)-3-methoxy-6-(7-methyl-3-methylene-oct-en-1-ynyl)-cyclohex-5-ene-1,2,4-triol.[4]

Phomentrioloxin showed interesting phytotoxic activities, e.g., reduced growth and chlorophyll content of fronds of *Lemna minor* and inhibited tomato rootlet elongation,[4] but showed no fungicidal, antibacterial, and zootoxic activities. SAR studies on different fungal metabolites, e.g., nonenolides (putaminoxins and stagonolides), cytochalasins, oxazatricycloalkalenones, alternethanoxins, and agropyrenol, a phytotoxic substituted salycilic aldehyde produced by different fungal species,[5−8] were carried out.

Considering the uncommon chemical structure of phomentrioloxin containing a 1,2,4-trihydroxy-3-methoxy cyclohexene ring and a polyunsaturated geranyl moiety, and its promising biological properties, it seemed worthwhile to further study this metabolite, trying to ascertain its potential as a novel natural herbicide and to identify the structural features essential for its biological activity. For this reason, seven derivatives (**2**−**8**, Figure 1) were prepared by chemical transformation of the functionalities present in **1** and tested for their phytotoxic, antimicrobial, and zootoxic activities.

MATERIALS AND METHODS

General Experimental Procedures. Infrared (IR) spectra were recorded as a deposit glass film on a Perkin-Elmer (Norwalk, CT, USA) spectrometer, and ultraviolet (UV) spectra were measured in MeCN, unless otherwise noted, on a Perkin-Elmer spectrophotometer. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded at 600 or 400 MHz in $CDCl_3$, on Bruker (Kalsruhe, Germany) spectrometers. The same solvent was used as the internal standard. Electrospray ionization (ESI) mass spectra (MS) were recorded on an Agilent Technologies (Milano, Italy) 6120 Quadrupole liquid chromatography mass spectrometry (LC/MS) instrument. Analytical and preparative thin layer chromatography (TLC) were performed on silica gel (Kieselgel 60, F_{254}, 0.25 and 0.5 mm, respectively) (Merck, Darmstadt, Germany) plates. The spots were visualized by exposure to UV radiation (254) or by spraying first with 10% H_2SO_4 in MeOH and then with 5% phosphomolybdic acid in EtOH, followed by heating at 110 °C for 10 min. Column chromatography was performed on a Kieselgel column, 60, 0.063−0.200 mm (3.0 × 85 cm) (Merck, Darmstadt, Germany).

Received: July 11, 2013
Revised: September 10, 2013
Accepted: September 15, 2013
Published: September 15, 2013

   dx.doi.org/10.1021/jf4030618 | J. Agric. Food Chem. 2013, 61, 9645−9649

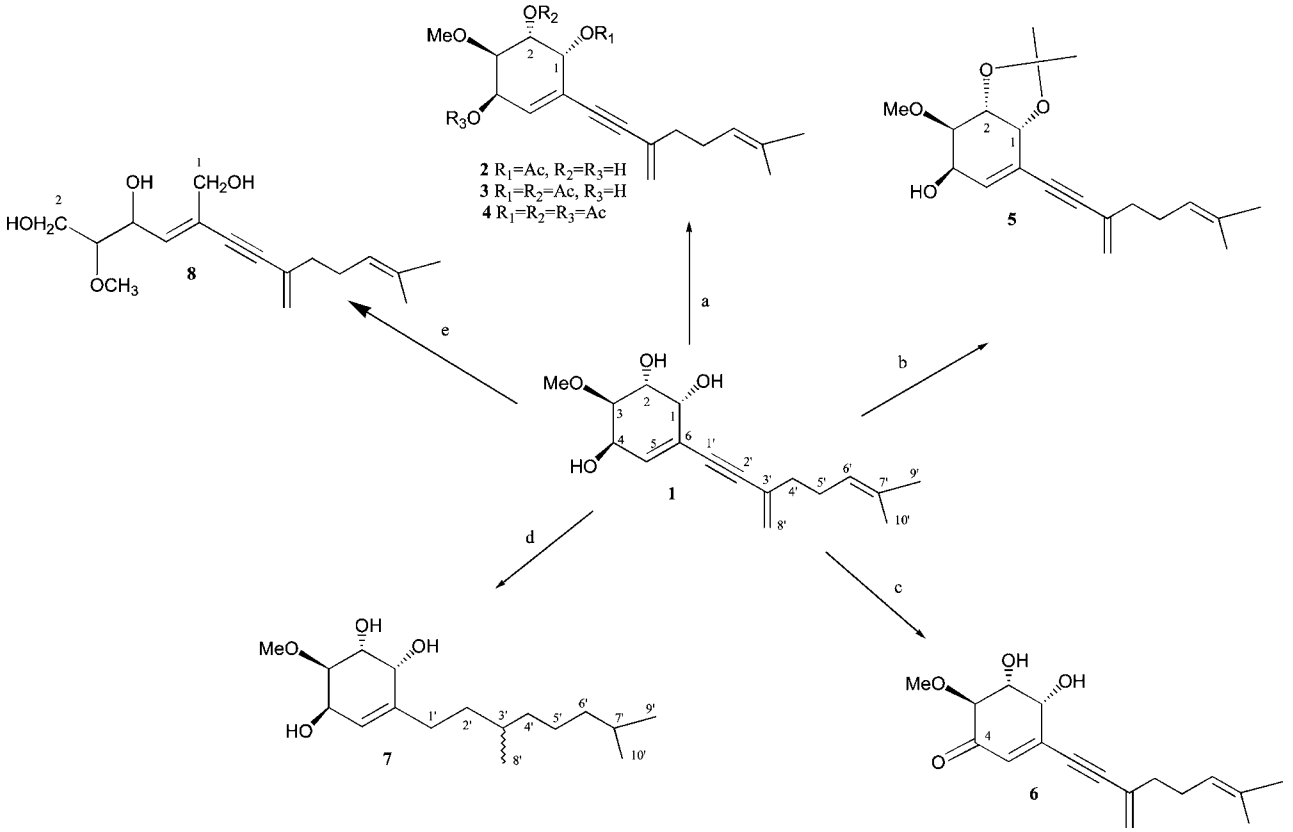

Figure 1. Structures of phomentrioloxin (**1**) and hemisynthesis and structures of its derivatives **2**−**8**. Reagents and conditions: (a) Ac_2O, pyridine, rt, 5 or 10 min; (b) dry Me_2CO, dry $CuSO_4$, rt, 2 h; (c) MnO_2, CH_2Cl_2, rt, 5 h; (d) H_2, 5% Rd/C, MeOH, rt, 15 min; (e) MeOH, $NaIO_4$ rt, 1 h; MeOH, $NaBH_4$ rt, 1 h.

Production, Extraction, and Purification of Phomentrioloxin (1). To produce phomentrioloxin (**1**), the fungus *Diaporthe gulyae* (anamorph *Phomopsis* sp.), previously isolated from naturally symptomatic saffron thistle (*C. lanatus*) plants in Australia, was used.[9] Pure cultures were maintained on potato-dextrose-agar (PDA) (Sigma-Aldrich Chemic GmbH, Buchs, Switzerland) and stored in the collection of the Istituto di Scienze delle Produzioni Alimentari, CNR, Italy, with the code ITEM13496. The fungus was grown on a mineral defined liquid media named M1-D[10] as previously reported.[4] The purification of the organic extract obtained from its culture filtrate (4 L), carried out as previously described,[4] produced phomentrioloxin (**1**) as an amorphous solid in amounts (40.3 mg, 10.0 mg/L) sufficient for the preparation of the derivatives.

1-*O*-Acetyl- and 1,2-*O*,*O*′-Diacetyl-phomentrioloxin (2 and 3). Compound **1** (12 mg) was acetylated with pyridine (80 μL) and Ac_2O (80 μL) at room temperature for 5 min. The reaction was stopped by the addition of MeOH, and the azeotrope, obtained by the addition of benzene, was evaporated by an N_2 stream. The oily residue (13.7 mg) was purified by preparative TLC eluted with $CHCl_3$/*i*-PrOH (97:3, v/v) to give the 1-*O*-acetyl and 1,2-*O*,*O*′-diacetyl derivatives of phomentrioloxin (**2** and **3**) as homogeneous compounds (R_f 0.20, 4.1 mg; R_f 0.40, 3.9 mg, respectively). Derivative **2** had IR ν_{max} 3382, 1714, 1661, 1646, 1615, 1269 cm^{-1}. UV λ_{max} nm (log ε): 274 (sh), 257 (3.13). ^1H NMR spectrum (Table 1); ESIMS (+) *m*/*z*: 373 $[M + K]^+$. 357 $[M + Na]^+$. Derivative **3** had IR ν_{max} 3397, 1721, 1661,1646, 1615, 1269 cm^{-1}. UV λ_{max} nm (log ε): 273 (sh), 261 (3.24). ^1H NMR spectrum (Table1); ESIMS (+) *m*/*z*: 415 $[M + K]^+$, 399 $[M + Na]^+$.

1,2,4-*O*,*O*′,*O*″-Triacetyl Derivative (4) and 1,2-*O*,*O*′-Isopropylidenephomentrioloxin (5). These two compounds were prepared by routine acetylation with acetic anhydride and pyridine, and by acid ketalization with dry Me_2CO of **1**, respectively, as previously reported.[4]

4′,*O*-Didehydrophomentrioloxin (6). Compound **1** (9 mg) dissolved in dry CH_2Cl_2 (3 mL) was oxidized with dry MnO_2 (65 mg), under stirring at room temperature for 5 h. The mixture was then filtered, and the solvent evaporated under reduced pressure to give an oily residue, which was purified by preparative TLC (silica gel, eluent $CHCl_3$/*i*-PrOH (95:5, v/v)) giving **6** as a homogeneous compound (R_f 0.56, 3 mg). Derivative **6** had IR ν_{max} 3385, 2186, 1674, 1607, 1594 cm^{-1}. UV λ_{max} nm (log ε): 283 (3.51). ^1H NMR (Table 1). ESIMS (+) *m*/*z*: 313 $[M + Na]^+$.

1′,1′,2′,2′,3′,8′,6′,7′-Octahydrophomentrioloxin (7). Compound **1** (8 mg) was first dissolved in MeOH (500 μL), then added to a presaturated 5% Rd/C (3 mg) suspension in the same solvent (500 μL), and hydrogenated at room temperature and atmospheric pressure under stirring conditions. The reaction was stopped after 15 min by filtration, and the solvent evaporated under reduced pressure; the residue (13.5 mg) was purified by preparative TLC (silica gel, eluent petroleum ether/acetone (7:3, v/v)) giving **7** as a homogeneous compound (3 mg, R_f 0.35). Derivative **7** had IR ν_{max} 3410, 1666, 1460 cm^{-1}. UV λ_{max} nm (log ε): < 220. ^1H NMR (Table 1). ESIMS (+) *m*/*z*: 339 $[M + K]^+$, 323 $[M + Na]^+$.

Conversion of Phomentrioloxin into 2-Methoxy-5-(3-methylene-oct-6-en-1-ynyl)-hex-4-ene-1,3,6-triol (8). Compound **1** (7 mg) dissolved in MeOH (10.2 mL) was oxidized with an aqueous solution (3.2 mL) of $NaIO_4$ (8 mg) under stirring at room temperature in the dark. After 1 h, ethylene glycol (0.6 mL) and cold acetone (3.2 mL) were added, and the precipitated $NaIO_3$ was removed by filtration. The solution was extracted with CH_2Cl_2 (4 × 15 mL), and the extract evaporated under reduced pressure. The residue, dissolved in MeOH (2 mL), was reduced with $NaBH_4$ (8 mg) for 1 h at room temperature. The reaction was stopped by neutralization with 1 M HCl (1 mL) and the aqueous solution extracted with CH_2Cl_2. The extract was dehydrated (Na_2SO_4) and purified by TLC (silica gel, eluent $CHCl_3$/*i*-PrOH (85:15, v/v)) giving **8** as a homogeneous compound (3 mg, R_f 0.57). Derivative **8** had IR ν_{max} 3390, 1643, 1615,

dx.doi.org/10.1021/jf4030618 | J. Agric. Food Chem. 2013, 61, 9645−9649

Table 1. ^{1}H NMR Data of Phomentrioloxin (1) and Its Derivatives (2, 3, and 6−8)[a]

	1	2	3	6	7	8
position	δ H (J in Hz)	δ H (J in Hz)	δ H (J in Hz)	δ H (J in Hz)	δ H (J in Hz)	δ H (J in Hz)
1	4.35 (1H) br s	5.70 (1H) d (4.1)	5.71 (1H) d (3.8)	4.56 (1H) br s	4.24 (1H) m[c]	4.28 (1H) br d (12.6) 4.19 (1H) br d (12.6)
2	4.21 (1H) ddd (7.9, 3.6, 2.0)	4.32 (1H) ddd (7.2, 4.1, 2.0)	5.56 (1H) dd (7.2, 3.8)	4.05 (1H) dd (9.2, 3.3)	4.24 (1H) m[c]	3.82 (1H) d (11.7) 3.77 (1H) d (11.7)
3	3.70 (1H) dd (7.9, 4.1)	3.66 (1H) dd (7.2, 4.4)	3.68 (1H) dd (7.2, 4.2)	4.00 (1H) d (9.2)	4.00 (1H) dd (10.0, 4.0)	3.29 (1H) m
4	4.51 (1H) ddd (6.1, 4.1, 4.0)	4.51 (1H) m	4.44 (1H) m		4.44 (1H) dd (5.3, 4.0)	4.73 (1H) dd (8.5, 6.6)
5	6.16 (1H) d (4.0)	6.18 (1H) d (3.6)	6.16 (1H) d (3.7)	6.21 (1H) s	5.71 (1H) d (5.3)	6.01 (1H) d (8.5)
6						
1′					[d]	
2′					[d]	
3′					[d]	
4′	2.22 (2H) m	2.13 (2H) m	2.15 (2H) m	2.24 (2H) m	[d]	2.20 (2H) m
5′	1.64 (2H) m	1.58 (2H) m	1.57 (2H) m	1.54 (2H) m	[d]	1.51 (2H) m
6′	5.13 m	5.11 (1H) m	5.07 (1H) m	5.10 (1H) m	[d]	5.11 (1H) m
7′					[d]	
8′	5.40 (1H) d (1.5) 5.31 (1H) d (1.5)	5.31 (1H) d (2.4) 5.25 (1H) d (2.4)	5.31 (1H) d (1.5) 5.25 (1H) d (1.5)	5.51 (1H) s 5.43 (1H) s	0.90 (3H) d (6.5)[c]	5.35 (1H) s 5.27 (1H) s
9′[b]	2.22 (3H) s	2.13 (3H) s	2.15 (3H) s	1.62 (3H) s	0.90 (3H) d (6.5)[c]	1.62 (3H) s
10′[b]	1.85 (3H) s	1.68 (3H) s	1.67 (3H) s	1.69 (3H) s	0.90 (3H) d (6.5)[c]	1.69 (3H) s
OMe	3.55 (3H) s	3.54 (3H) s	3.55 (3H) s	3.64 (3H) s	3.54 (3H) s	3.48 (3H) s
HO-1	2.65 (1H) d (1.4)			2.82 (1H) s		
HO-2	2.68 (1H) d (2.0)	2.54 (1H) d (2.0)				
HO-4	2.64 (1H) d (6.1)	2.56 (1H) d (2.0)	2.62 (1H) d (2.0)			
MeCO		2.15 (3H) s	2.08 (3H) s, 2.07 (3H) s			

[a]The chemical shifts are in δ values (ppm) from TMS. [b]These assignments could be reversed. [c]The signals were overlapped. [d]These signals are observed as complex multiplets integrating for 12 protons in the region of δ 2.0−1.0.

1573 cm^{-1}. UV λ_{max} nm (log ε): 274 (sh); 256 (3.15), 256 (3.15). ^{1}H NMR (Table 1); ESIMS (+) *m/z*: 317 $[M + Na]^+$.

Biological Assays. A number of assays on different organisms were performed in order to characterize prelimiarly the biological properties of the derivatives compared to the source main metabolite **1** and thus to allow a structure−activity relationship study. Each metabolite was first dissolved in a minimum amount of MeOH (10^{-1} M or not higher than 2% in the final solutions) and then diluted with distilled water to the desired concentrations. The following bioassays were performed.

Leaf Disk Puncture Assay. All of the compounds (**1**−**8**) were tested by using a leaf puncture assay on 4 plant species, namely, *Chenopodium album* L., *Mercurialis annua* L., *Sonchus oleraceus* L., and *Carthamus lanatus* L. Pure compounds were tested at 6.85×10^{-3} M by applying a droplet (20 μL−2% solution in MeOH) of solution to detached leaves previously punctured with a needle. Five replications (droplets) on separate leaves were used for each metabolite and for each plant species tested. Leaves were kept in a moistened chamber under continuous fluorescent lights. Symptoms were estimated visually between 3 and 5 days after droplet application, by using a visual scale from 0 (no symptoms) to 4 (necrosis wider than 1 cm). Control treatments were carried out by applying droplets not containing the metabolites.

Chlorophyll Degradation. Phomentrioloxin and derivatives were tested on the aquatic plant *Lemna minor* for the possible capability to degrade chlorophyll. The bioassay was carried out according to the protocol already described in detail.[4] All of the compounds were tested at 6.85×10^{-3} M.

Antimicrobial Bioassay. The antimicrobial activity was tested against three microorganisms by using an agar diffusion assay according to the protocol already described.[11] In particular, the antifungal activity was tested on *Geotrichum candidum* grown on PDA, whereas the antibiotic activity was assayed against *Bacillus subtilis* (a gram positive bacterium) grown on TGYA (tryptic glucose yeast agar) (Biolife, Bothell, WA) and *Escherichia coli* (a gram negative bacterium), grown on LB agar (Sigma, St. Louis, MO, USA). Up to 60 μg of each metabolite was applied per diskette. Three replications were performed for each compound. The eventual presence of an inhibition halo of the microbial growth was visually assessed 1 day after the application.

Zootoxic Activity Assay. The zootoxic activity was evaluated on *Artemia salina* L. larvae (brine shrimps) by using the protocol already described.[11] The metabolites were tested at 1.7×10^{-4} M, with four replications for each compound. Forty-eight hours after performing the assay, the number of dead larvae was counted, and toxicity was then expressed as percentage of dead larvae in reference to the total.

Data Analysis. All of the bioassays were performed twice with at least 3 replicates. When appropriate, standard deviation was determined.

RESULTS AND DISCUSSION

Phomentrioloxin (**1**) was recently isolated as a new phytotoxic geranylhydroquinone from *D. gulyae* culture filtrates. It was characterized as (1*S*,2*S*,3*S*,4*S*)-3-methoxy-6-(7-methyl-3-methylene-oct-en-1-ynyl)-cyclohex-5-ene-1,2,4-triol using spectroscopic and chemical methods combined with X-ray analysis.[4] In particular, the application of the advanced Mosher's method[12] allowed assignment of the absolute configuration to phomentrioloxin as reported below. However, due to a mistake in the application of these rules this absolute configuration must be corrected to 1*R*,2*R*,3*R*,4*R*.

Seven phomentrioloxin derivatives were prepared in order to determine the relationships between the structure of phomen-

dx.doi.org/10.1021/jf4030618 | J. Agric. Food Chem. 2013, 61, 9645−9649

Table 2. Biological Assays of Phomentrioloxin (1) and Its Derivatives (2−8)

bioassay	leaf puncture assay[a]				chlorophyll[b]	protoplasts[c]	antibiosis[d]			zootoxicity[e]
species	*C. lanatus*	*S. oleraceus*	*M. annua*	*C. album*	*L. minor*	*A. thaliana*	*G. candidum*	*B. subtilis*	*E. coli*	*A.salina*
compd										
1	3	4	2	1	1.79 ± 0.30	60.3 ± 6	-	-	-	0
2	4	3	2	1	4.32 ± 0.54	41.3 ± 14	-	-	-	84
3	0	0	0	0	4.85 ± 0.46	80.3 ± 8	-	-	-	0
4	0	0	0	0	4.33 ± 0.02	83.0 ± 4	-	-	-	0
5	1	1	1	0	4.70 ± 0.62	71.0 ± 4	-	-	-	0
6	0	0	0	0	4.27 ± 0.80	84.0 ± 12	-	-	-	0
7	0	1	1	2	2.08 ± 0.14	48.0 ± 3	-	-	-	0
8	0	0	0	0	4.09 ± 0.31	83.0 ± 5	-	-	-	0

[a]Empiric scale from 0 (= inactive) to 4 (necrosis around 1 cm diameter). [b]Total chlorophyll ± SD; control = 4.15 ± 0.35. [c]Protoplast viability (% of the total); control = 85.3 ± 11.02. [d]Inhibition halo: -, absence. [e]Larva mortality after 48 h of exposure (% of the total).

trioloxin and its biological properties to identify the active sites of the compound and, possibly, increase or change its phytotoxicity. As previously reported,[4] **1** was converted into the corresponding 1,2,4-*O*,*O*′,*O*″-triacetyl derivative (**4**, Figure 1) by routine acetylation carried out with acetic anhydride and pyridine, which showed the reversible modification of the 1,2,4-triol system of the cyclohexene moiety. When the same reaction was carried out for a shorter time (5 min), two partially acetylated derivatives were obtained as the 1-*O*-acetyl and 1,2-*O*,*O*′-diacetylphomentrioloxin (**2** and **3**, Figure 1). A different modification of the same diol system was obtained by conversion of **1** into the corresponding 1,2-*O*,*O*′-isopropylidene derivative (**5**, Figure 1). Derivative **5** was obtained by the reaction of **1** with dry Me_2CO and dry $CuSO_4$. A further different modification of the cyclohexentriol moiety was obtained by selective oxidation to a ketone of the secondary hydroxy group C-4 of **1** with MnO_2, obtaining derivative **6**. Derivative **6** also showed a marked modification of the stereochemistry of the cyclohexene ring. Several attempts were made using different catalysts, solvents, and time of reaction to partially or totally hydrogenate the triple and the two double bonds of the side chain at C-6. These reactions aimed at differently modifying 7-methyl-3-methylene-oct-6-ene-1-ynyl and at having responses on the role of its functionalities on the biological activity. The best results were obtained using 5% Rd/C in MeOH at atmospheric pressure and at room temperature. The main derivative obtained was 1′,1′,2′,2′,3′,8′,6′,7′-octahydro derivative (**7**), as the other partially hydrogenated compounds were present in very low yield in the reaction mixture. Compound **7** showed the complete saturation of the side chain at C-6 that became a 3,7-dimethyloctyl. Finally, the reductive opening of the cyclohexentriol moiety was obtained by the selective oxidation cleavage with $NaIO_4$ of the diol system present between C-1 and C-2. The corresponding unstable dialdehyde was immediately reduced with $NaBH_4$ to the corresponding primary hydroxy groups yielding derivative **8**. The latter, 2-methoxy-5-(3-methylene-oct-6-en-1-ynyl)-hex-4-ene-1,3,6-triol (**8**), had a structure completely different from that of the parent compound, except for the part corresponding to the side chain. The structures of all of the derivatives **2**, **3** and **6**−**8** were determined by comparing their spectroscopic data, essentially IR, ^{1}H NMR (see Table 1), and ESIMS with those of phomentrioloxin.

In particular, the IR spectrum of **2** and **3** differed from that of **1** for the presence of the carbonyl and ester groups at 1714 and 1269, and 1721 and 1269 cm^{-1}, respectively. The ^{1}H NMR spectrum of **2** (Table 1) differed from that of **1** for the downfield shift ($\Delta\delta$ 1.35) of HC-1, which now appeared as a doublet (J = 4.1 Hz) at δ 5.70, the presence of the singlet of the acetyl group at δ 2.15, and the absence of the HO-1 signal. The ^{1}H NMR spectrum of **3** (Table 1) differed from that of **1** for the downfield shift of both HC-1 and HC-2 ($\Delta\delta$ 1.36 and 1.21) appearing as a doublet (J = 3.8 Hz) and a double doublet (J = 7.2 and 3.8 Hz) at δ 5.71 and 5.56, respectively. Furthermore, the presence of the two singlets due to the two acetyl groups was observed at δ 2.08 and 2.07, while the HO-1 and HO-2 signals were absent. Their ESIMS spectra showed the potassium $[M + K]^+$ and sodium $[M + Na]^+$ clusters at m/z 373 and 357, and 415 and 399, respectively. The IR spectrum of the oxidized derivative **6** differed from that of **1** essentially due to the presence of the band of the conjugated carbonyl group at 1674 cm^{-1}. Its ^{1}H NMR spectrum (Table 1) differed from that of **1** essentially because of the absence of the HO-4 signal, while H-5 appeared as a singlet at δ 6.21. Its ESIMS spectrum showed the sodium cluster $[M + Na]^+$ at m/z 313. The IR spectrum of the octahydroderivative **7** differed from that of **1** for the absence of the signal of the alkyne group. Its ^{1}H NMR spectrum (Table 1), compared to that of **1**, showed the presence of one olefinic signal due to H-5 and the presence of three doublets (J = 6.5 Hz) due to methyl group Me-8′, Me-9′, and Me-10′, which gave an overlapped signal at δ 0.90. Its ESIMS showed the sodium cluster $[M + Na]^+$ at m/z 317. Finally, the IR spectrum of derivative **8** did not substantially differ from that of **1**, while significant differences were observed comparing their ^{1}H NMR spectra (Table 1). In particular, the presence of two broad doublets (J = 12.6 Hz) and two doublets (J = 11.7 Hz), typical AB systems of the two CH_2O-1 and CH_2O-2, resonated at δ 4.28 and 4.19, and 3.82 and 3.77, respectively. Its ESIMS spectrum showed the sodium cluster $[M + Na]^+$ at m/z 317.

Assayes on leaves by puncture confirmed that **1** and **2** were equally toxic, causing the appearance of wide necrosis (Table 2); compounds **5** and **7** caused the appearance of necrosis of modest size, whereas all of the other compounds proved to be inactive. On protoplasts (60.3% viable protoplast obtained testing **1**), only **2** and **7** proved to be active, leaving 41.3 and 48.0% viable protoplasts after the treatment, respectively (viability of the control 85.3%). When assayed on *L. minor* for chlorophyll degradation, besides **1**, only compound **7** proved to be effective, causing around 50% chlorophyll degradation, whereas all of the other compounds were inactive.

The results obtained confirmed the preliminary ones obtained testing **1**, **4**, and **5**.[4] Furthermore, they showed that

dx.doi.org/10.1021/jf4030618 | J. Agric. Food Chem. 2013, 61, 9645−9649

the hydroxy groups at C-2 and C-4 are very important features for phytotoxicity. In fact, when the 2-hydroxy or the 2- and 4-hydroxy groups were acetylated as in **3** and **4**, respectively, a total loss of activity was observed. The role of these two groups (2- and 4-hydroxy) was confirmed by the reduced activity observed when the hydroxy group at C-2, together with that at C-1, was ketalized as in **5** and by the total loss of activity when the hydroxy group at C-4 was oxidized as in **6**. The hydroxy group at C-1 seems not to be important for activity. In fact, when it was acetylated as in **2**, this derivative proved to be as toxic as **1**. The reduced activity showed by derivative **7** indicated that the unsaturation of the geranyl side chain also plays a role in imparting phytotoxicity. Finally, the lack of activity of derivative **8** also meant that the presence of an unchanged cyclohexenetriol ring is important for activity.

When assayed on brine shrimps at 1.7×10^{-4} M, only compound **2** caused 45% larvae mortality after 24 h of exposure to the toxin, which increased to 84% at 48 h. All the other compounds were inactive at the same concentration. The activity of derivative **2** could indicate that it can more easily penetrate the cell membrane due to its increased lipophilicity. Probably, the conversion of acetyl derivative **2** into the parent compound occurs at physiological pH as frequently observed in other natural metabolites and is known as lethal metabolism.[13] This may not occur completely for the analogues diacetyl and triacetyl derivatives **3** and **4**. None of the compounds was toxic when assayed on Gram+ and Gram− bacteria, and *G. candidum* using concentrations up to 60 μg/diskette.

These results could be useful when studying the mode of action of phomentrioloxin and in devising new natural products with potential application as herbicides in agriculture. Indeed, although the monoacetyl derivatives (**2**) has the same level of phytotoxicity of phomentrioloxin, this latter remains the best potential herbicide as **2** exhibits a significant increase of zootoxicity.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

IR, UV, 1D ^{1}H NMR, and HRESI MS spectra of phomentrioloxin derivatives (**2**−**8**). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +39 081 2539178. Fax: +39 081 6741330. E-mail: evidente@unina.it.

Funding

C.T. is supported by a scholarship from Charles Sturt University, Australia. A.E. is associated with the Istituto di Chimica Biomolecolare, CNR, Pozzuoli, Italy.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

NMR spectra were recorded by Dominique Melck in the laboratory of Istituto di Chimica Biomolecolare del CNR, Pozzuoli, Italy.

■ REFERENCES

(1) Shorten, C. Note Number LC0225. http://www.dpi.vic.gov.au/agriculture/pests-diseases-and-weeds/weeds/other-declared-weeds/saffron-thistle (accessed in 2013).

(2) Ash, G. J.; Stodart, B.; Sakuanrungsirikul, S.; Anschaw, E.; Crump, N.; Hailstones, D.; Harper, D. I. Genetic characterization of a novel *Phomopsis* sp., a putative biocontrol agent for *Carthamus lanatus*. *Mycologia* **2010**, *102*, 54−61.

(3) Thompson, S. M.; Tan, Y. P.; Young, A. J.; Neate, S. M.; Aiteken, A. A. B.; Shivvas, R. G. Stem cankers on sunflower (*Helianthus annuus*) in Australia reveal a complex of pathogenic *Diaporthe* (*Phomopsis*) species. *Persoonia* **2011**, *27*, 80−99.

(4) Cimmino, A.; Andolfi, A.; Zonno, M. C.; Troise, C.; Santini, A.; Tuzi, A.; Vurro, M.; Ash, G.; Evidente, A. Phomentrioloxin: a phytotoxic pentasubstituted geranylhexentriol produced by *Phomopsis* sp., a potential mycoherbicide for *Carthamus lanatus* biocontrol. *J. Nat. Prod.* **2012**, *75*, 1130−1137.

(5) Evidente, A.; Capasso, R.; Andolfi, A.; Vurro, M.; Zonno, M. C. Structure-activity relationships studies of putaminoxins and pinolidoxins phytotoxic nonenolides produced by phytopathogenic *Phoma* and *Ascochyta* species. *Nat. Toxins* **1998**, *6*, 183−188.

(6) Evidente, A.; Andolfi, A.; Cimmino, A. Fungal phytotoxins for control of *Cirsium arvense* and *Sonchus arvensis*. *Pest Technol.* **2011**, *5*, 1−17 and references therein cited.

(7) Evidente, A.; Punzo, B.; Andolfi, A.; Berestetskiy, A.; Motta, A. Alternethanoxins A and B, polycyclic ethanones produced by *Alternaria sonchi*, potential mycoherbicides for *Sonchus arvensis* biocontrol. *J. Agric. Food Chem.* **2009**, *57*, 6656−6660.

(8) Cimmino, A.; Zonno, M. C.; Andolfi, A.; Troise, C.; Motta, A.; Vurro, M.; Evidente, A. Agropyrenol, a phytotoxic fungal metabolite, and its derivatives: A structure-activity relationship study. *J. Agric. Food Chem.* **2013**, *61*, 1779−1783.

(9) Crump, N. S.; Ash, G. J.; Nickandrow, A. *Phomopsis* spp. associated with die-back of saffron thistle (*Carthamus lanatus*). *Australas. Plant Pathol.* **1996**, *25*, 143.

(10) Pinkerton, F.; Strobel, G. A. Serinol as an activator of toxin production in attenuated cultures of *Helminthosporium sacchari*. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 4007−4011.

(11) Bottalico, A.; Capasso, R.; Evidente, A.; Randazzo, G.; Vurro, M. Cytochalasins structure-activity relationship. *Phytochemistry* **1990**, *29*, 93−96.

(12) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. High-field FT NMR application of Mosher's method. The absolute configuration of marine terpenoids. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.

(13) Hassal, K. A. *Biochemistry and Uses of Pesticides*; Verlag Chemie: Weinheim, Germany, 1990; pp 58, 72, 304, 429, and 497.