JOURNAL OF NATURAL PRODUCTS

Article

Cite This: J. Nat. Prod. 2017, 80, 3296−3304

pubs.acs.org/jnp

Eudesmane Sesquiterpenes from *Verbesina lanata* with Inhibitory Activity against Grapevine Downy Mildew

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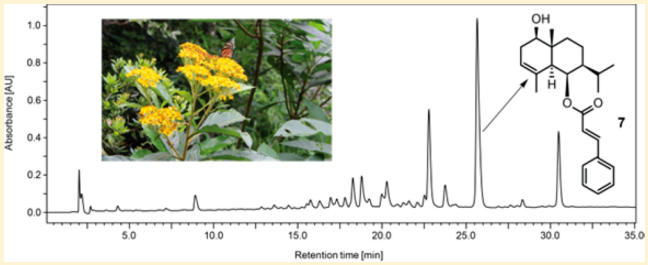
Supporting Information

ABSTRACT: An in-house library of more than 3000 extracts of plant and fungal origin was screened against some major plant pathogens. As one of the hits, an ethyl acetate extract from inflorescences of *Verbesina lanata* showed significant inhibitory activity in vitro against grapevine downy mildew (*Plasmopara viticola*), with a MIC_{100} value of 35 μg/mL. An emulsifiable concentrate formulation with 50 mg/g of the extract was developed for in vivo evaluation. A suspension of the formulation containing 1 mg/mL of extract lowered leaf surface infection of grapevine seedling by 82% compared to the nontreated control. With the aid of HPLC-based activity profiling, the antifungal activity was correlated with a series of lipophilic compounds. Preparative isolation by a combination of chromatographic techniques afforded 16 eudesmane sesquiterpenes including eight new congeners. Nine compounds were obtained in sufficient quantities to be tested in vitro and were found to inhibit the zoospore activity of *P. viticola* with MIC_{100} values ranging from 4 to 50 μg/mL. The two major compounds, 6β-cinnamoyloxy-4β,9β,15-trihydroxyeudesmane (**9**) and 6β-cinnamoyloxy-1β,15-dihydroxyeudesm-4-en-3-one (**13**), showed MIC_{100} values of 5 and 31 μg/mL, respectively.



Plasmopara viticola (Berk. & M. A. Curtis) Berl. & de Toni, known as grapevine downy mildew, is an obligately biotrophic oomycete that infects all green parts of grapevine plants. The sporangia are spread by windblown rain. Under weather conditions favorable for the pathogen, up to 100% of the yield can be lost if plants are left untreated.[1]

Copper salts are widely used to fight against a wide range of plant pathogens.[2] Their use in agriculture was established in the 1880s when the French scientist Millardet demonstrated that spraying vineyards with a mixture of copper sulfate, lime, and water (Bordeaux mixture) drastically reduced infection of grapevine by downy mildew.[3] The use of copper is still permitted in conventional and organic production systems,[4] but its utilization is increasingly criticized due to an unfavorable ecotoxicological profile.[2,5,6] Copper accumulates in soils and is potentially toxic to some nontarget organisms.[7−10]

Natural products, especially plant extracts, could serve as sustainable and environmentally friendly alternatives since they are typically rapidly degraded under field conditions.[11−15] Promising plant extracts with reported activity against *P. viticola* include *Yucca schidigera*, *Salvia officinalis*, *Inula viscosa*, *Glycyrrhiza glabra*, *Larix decidua*, *Juncus effusus*, and *Vitis vinifera*.[4,14,16−20]

In an ongoing search for safer replacements of copper fungicides, an in-house library comprising over 3000 extracts of plant and fungal origin (10 mg/mL in DMSO) was screened for in vitro inhibitory activity against grapevine downy mildew.[20,21] As one of the hits, an ethyl acetate extract from inflorescences of *Verbesina lanata* B. L. Rob. & Greenm. (Asteraceae) showed pronounced activity (MIC_{100} of 35 μg/mL). The genus *Verbesina*, commonly known as "crownbeard", comprises over 300 species of herbs, shrubs, and trees bearing numerous bright yellow flowerheads.[22−24] *V. lanata* is distributed in Central America[22] and has not been phytochemically investigated up to now.

We here report on the isolation and structure elucidation of 16 eudesmane sesquiterpenes (**1**−**16**), including eight new congeners, with strong inhibitory activity against *P. viticola*.

RESULTS AND DISCUSSION

Compound Isolation and Structure Elucidation. The ethyl acetate extract of *V. lanata* flowerheads was fractionated by silica gel column chromatography. Out of a total of 26

Received: October 17, 2017
Published: December 4, 2017

DOI: 10.1021/acs.jnatprod.7b00868
J. Nat. Prod. 2017, 80, 3296−3304

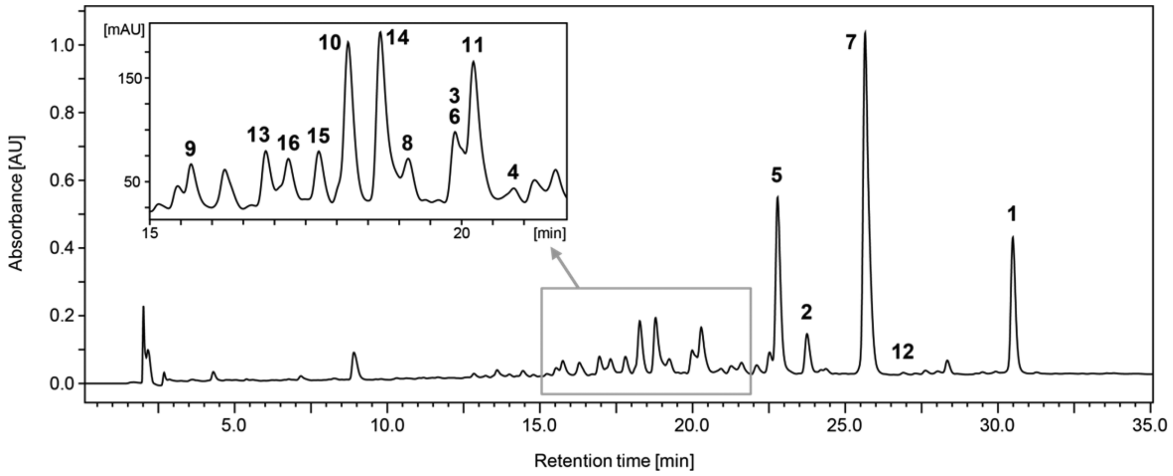


Figure 1. HPLC-PDA analysis of the ethyl acetate extract of *V. lanata*. SunFire C_{18} column; A: water + 0.1% formic acid, B: acetonitrile + 0.1% formic acid; 30% to 100% B in 30 min, and 100% B for 5 min; detection at 254 nm. Bolded numerals refer to isolated compounds.

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for Compounds 9−12 ($CDCl_3$; 500 MHz for ^{1}H, 125 MHz for ^{13}C; δ in ppm)

	9		10		11		12[a]	
position	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type
1	2.01, dt (12.8, 2.8), β	39.5, CH_2	3.85, dd (11.9, 4.3), α	75.1, CH	3.11, d (9.2), α	84.6, CH	3.41, dd (11.6, 4.0), α	78.2, CH
	1.16, m,[b] α							
2	1.69, m[b]	20.8, CH_2	1.98, ddd (13.7, 4.3, 2.8), α	37.1, CH_2	3.61, ddd (11.0, 9.2, 5.7), β	70.5, CH	1.66, m[b]	26.7, CH_2
	1.69, m[b]		1.73, m,[b] β				1.66, m[b]	
3	1.70, m[b], β	35.8, CH_2	4.33, br m, β	74.4, CH	2.62, dd (13.2, 5.7), β	43.1, CH_2	2.17, m, α	32.5, CH_2
	1.54, td (14.0, 4.2), α				2.11, m[b], α		2.09, m,[b] β	
4		73.6, C		147.4, C		142.8, C		132.4, C
5	1.31, br s, α	49.0, CH	2.48, br s, α	46.5, CH	1.99, br s, α	51.9, CH		131.7, C
6	5.71, br s, α	70.8, CH	5.74, br s, α	71.3, CH	5.79, br s, α	70.9, CH	6.13, br d (1.8), α	71.2, CH
7	1.10, m[b], α	49.8, CH	1.17, m, α	50.4, CH	1.13, m, α	50.2, CH	1.06, m, α	48.8, CH
8	1.89, qd (11.6, 3.5), β	26.4, CH_2	1.72, m,[b] α	20.6, CH_2	1.68, dq (13.0, 2.8), α	20.2, CH_2	1.73, m[b]	20.4, CH_2
	1.61, m,[b] α		1.60, m, β		1.58, qd (13.0, 3.4), β		1.73, m[b]	
9	3.21, dd (11.6, 3.5), α	80.3, CH	2.04, dt (13.1, 3.5), β	37.3, CH_2	2.10, m[b], β	37.5, CH_2	2.07, m,[b] β	38.5, CH_2
			1.30, td (13.1, 3.5), α		1.24, td (13.0, 3.4), α		1.19, m,[b] α	
10		39.4, C		40.7, C		39.5, C		38.3, C
11	1.45, m	28.8, CH	1.36, m	28.2, CH	1.38, m	28.1, CH	1.58, m	29.0, CH
12	0.87, d (6.7)	20.6, CH_3	1.04, br d (6.5)	22.1, CH_3	0.87, d (6.7)	20.5, CH_3	0.96, d (7.3)	20.7, CH_3
13	0.93, d (6.7)	21.6, CH_3	0.87, br d (6.5)	20.5, CH_3	1.04, d (6.7)	22.1, CH_3	0.94, d (7.3)	20.7, CH_3
14	1.38, s	14.3, CH_3	1.00, s	12.5, CH_3	1.04, br s	14.4, CH_3	1.14, br s	18.3, CH_3
15	3.78, d (10.4)	69.0, CH_2	5.03, br s	112.8, CH_2	4.87, br s	110.9, CH_2	1.88, s	19.3, CH_3
	3.60, d (10.4)		4.88, br s		4.78, br s			
1′		167.5, C		167.0, C		166.9, C		165.7, C
2′	6.41, d (16.0)	118.5, CH	6.41, d (16.0)	118.5, CH	6.39, d (15.9)	118.4, CH	6.40, d (16.2)	118.8, CH
3′	7.67, d (16.0)	145.5, CH	7.70, d (16.0)	145.2, CH	7.68, d (15.9)	145.2, CH	7.65, d (16.2)	144.1, CH
4′		134.4, C		134.4, C		134.4, C		134.3, C
5′, 9′	7.51, m	128.4, CH	7.53, m	128.3, CH	7.51, m	128.2, CH	7.51, m	127.8, CH
6′, 8′	7.36, m[b]	129.0, CH	7.39, m[b]	129.0, CH	7.36, m[b]	129.0, CH	7.36, m[b]	128.6, CH
7′	7.36, m[b]	130.6, CH	7.39, m[b]	130.5, CH	7.36, m[b]	130.5, CH	7.36, m[b]	129.8, CH

[a] ^{13}C NMR data extracted from $^{1}H-^{13}C$ 2D inverse-detected experiments. [b] Overlapping signals.

fractions, five fractions showed strong antifungal activity against *P. viticola* in vitro (data not shown). HPLC microfractionation combined with bioactivity assessment in a process referred to as HPLC-based activity profiling[21] enabled the activity to be correlated with a group of peaks (Figure S1, Supporting Information) showing strong UV absorption maxima at 280 nm. Targeted isolation by a combination of preparative and semipreparative HPLC afforded compounds **1**−**16**, which were shown to account for most of the peaks in the HPLC-UV chromatographic trace (Figure 1).

Compounds **1**−**8** were identified by NMR spectroscopic data analysis and comparison with literature values. Compounds **1**−

DOI: 10.1021/acs.jnatprod.7b00868
J. Nat. Prod. 2017, 80, 3296−3304

8 were found to be known eudesmane sesquiterpenes with cinnamoyloxy groups attached at C-6. They were identified as 6*β*-cinnamoyloxy-4*β*-hydroxyeudesmane (**1**),[25] 6*β*-cinnamoyloxy-3*β*,4*α*-dihydroxyeudesmane (**2**),[26] 6*β*-cinnamoyloxy-3*α*-hydroperoxy-1*β*-hydroxyeudesm-4(15)-ene (**3**),[27] 6*β*-cinnamoyloxy-3*α*-hydroperoxy-1*β*-hydroxyeudesm-4-ene (**4**),[27] 6*β*-cinnamoyloxy-1*β*-hydroxyeudesm-4-en-3-one (**5**),[27] 6*β*-cinnamoyloxy-1*β*,3*β*-dihydroxyeudesm-4-ene (**6**),[26] 6*β*-cinnamoyloxy-1*β*-hydroxyeudesm-3-ene (**7**),[27] and 7-epi-6*α*-cinnamoyloxy-1*β*,4*α*-dihydroxyeudesmane (**8**).[26] These compounds have been previously isolated from species of the genus *Verbesina* and/or from *Brintonia discoidea*, but they are reported here for the first time from *V. lanata*. Some minor inconsistencies were detected in the previously reported NMR data, and some assignments were therefore revised. Full ^{1}H and ^{13}C NMR spectroscopic assignments of **1**−**8** are provided as Supporting Information.

Compound **9** was obtained as a pale yellow oil. Its molecular formula was established as $C_{24}H_{34}O_5$ from the $[M + Na]^+$ sodium adduct ion at *m*/*z* 425.2297 (calcd for $C_{24}H_{34}NaO_5^+$, 425.2299) in the HRESIMS and corroborated by the ^{13}C NMR data. The ^{13}C NMR spectrum showed the presence of signals for three methyls, five methylenes (including one oxygenated carbon (δ_C 69.0)), 12 methines (including seven olefinic and aromatic carbons), one oxygenated tertiary carbon (δ_C 73.6), one carbonyl (δ_C 167.5), and two quaternary carbons (δ_C 39.4 and 134.4). The ^{1}H and ^{13}C NMR data were fully assigned by 2D NMR ($^{1}H-^{1}H$, COSY, HSQC, and HMBC) experiments (Table 1). Comparison of the NMR data with those of compounds **1** and **2** indicated that **9** also possesses a *trans*-decalin eudesmane skeleton with an axial (*β*) cinnamoyloxy substituent at C-6. A hydroxy group was located at C-9 (δ_H 3.21, δ_C 80.3), as shown by a COSY correlation between H_2-8 (δ_H 1.89 and 1.61) and H-9. HMBC correlations of H-5 (δ_H 1.31) and H_2-15 (δ_H 3.78 and 3.60) to δ_C 73.6 allowed the assignment of the oxygenated tertiary carbon as C-4 (δ_C 73.6). By HMBC correlations of H_2-15 (δ_H 3.78 and 3.60) to C-3 (δ_C 35.8), C-4 (δ_C 73.6), and C-5 (δ_C 49.0), the position of the oxygenated methylene (δ_C 69.0) was assigned at C-15. The relative configuration was established from $^{1}H-^{1}H$ coupling constants and NOESY correlations (Figure 2). Thus, the

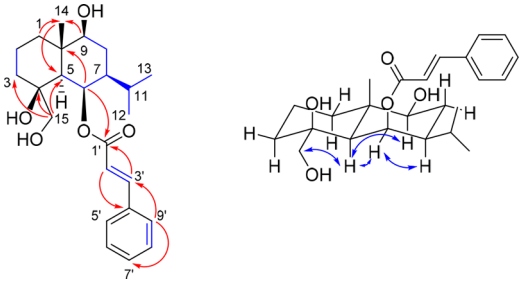
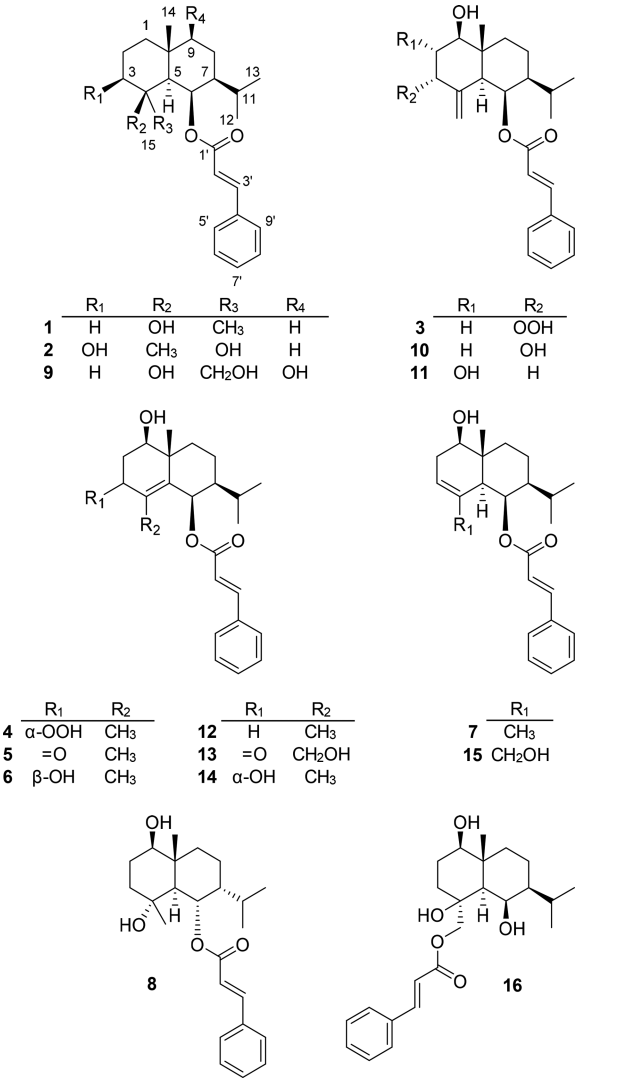


Figure 2. Key COSY (blue bonds), HMBC (red arrows), and NOESY correlations (blue arrows) of compound **9**.

hydroxy group at C-9 was determined as equatorial due to the diaxial coupling of H-9 (δ_H 3.21, dd, *J* = 11.6/3.5 Hz) with H-8$_{ax}$ (δ_H 1.89, qd, *J* = 11.6/3.5 Hz). A NOESY correlation of H-9 and H-5 (δ_H 1.31) confirmed the alpha-orientation of H-9. Similarly, the orientation of C-15 (δ_H 3.78 and 3.60) was assigned as alpha by the NOESY correlation of H_2-15 and H-5 (δ_H 1.31). Thus, the structure of **9** was established as 6*β*-cinnamoyloxy-4*β*,9*β*,15-trihydroxyeudesmane.



Compound **10** was obtained as a pale yellow, amorphous solid. It had a molecular formula of $C_{24}H_{32}O_4$ as determined from a $[M + Na]^+$ ion at *m*/*z* 407.2192 in the HRESIMS (calcd for $C_{24}H_{32}NaO_4^+$, 407.2193) and thus differed by 16 units from that of **3**. The NMR data of **10** (Table 1) closely resembled those of **3**. The only remarkable difference was an upfield shift of C-3 in **10** (δ_H 4.33, δ_C 74.4; vs δ_H 4.44, δ_C 87.0 in **3**), which suggested the replacement of the peroxide at C-3 by a hydroxy group. Key NMR correlations for **10** are available as Supporting Information (Figure S24). Accordingly, the compound was assigned as 6*β*-cinnamoyloxy-1*β*,3*α*-dihydroxyeudesm-4(15)-ene.

Compound **11**, isolated as a yellow oil, had the same molecular formula ($C_{24}H_{32}O_4$) as **10**, as determined by the HRESIMS $[M + H]^+$ ion at *m*/*z* 385.2373 (calcd for $C_{24}H_{33}O_4^+$, 385.2373). The NMR data indicated that **11** is a positional isomer of the latter. Comparison of ^{1}H, ^{13}C, and 2D NMR data (Table 1) suggested that the hydroxy group in **11** had to be located C-2. This assignment was corroborated by key COSY correlations of H-2 (δ_H 3.61) with H-1 (δ_H 3.11) and H_2-3 (δ_H 2.62 and 2.11). The *α*-equatorial orientation of OH-2 was deduced from the diaxial coupling of H-2 with H-1 and H-3 ($J_{H_1-H_2}$ = 9.2 Hz, and $J_{H_2-H_3}$ = 11.0 Hz, respectively) and confirmed by the NOESY correlation of H_3-14 with H-2ax (Figure S32, Supporting Information). Therefore, the structure

DOI: 10.1021/acs.jnatprod.7b00868
J. Nat. Prod. 2017, 80, 3296−3304

Table 2. ^{1}H and ^{13}C NMR Spectroscopic Data for Compounds 13−16 ($CDCl_3$; 500 MHz for ^{1}H, 125 MHz for ^{13}C; δ in ppm)

	13[a]		14		15		16	
position	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type
1	3.79, dd (10.1, 7.6), α	74.5, CH	3.70, dd (13.0, 3.5), α	73.5, CH	3.54, dd (10.0, 6.1), α	76.4, CH	3.22, dd (12.0, 3.5), α	79.7, CH
2	2.62, m[b]	42.5, CH_2	1.89, td (13.0, 4.0), β	36.0, CH_2	2.33, m[b]	31.9, CH_2	2.01, qd (12.0, 3.5), β	26.4, CH_2
	2.62, m[b]		1.82, ddd (13.0, 3.5, 1.7), α		2.00, m[b]		1.67, m, α	
3		198.5, C	4.06, br d (4.0), β	71.0, CH	5.63, br m	124.4, CH	1.78, dd (12.0, 3.5), β	34.2, CH_2
							1.63, m,[b] α	
4		136.2, C		132.9, C		136.6, C		73.8, C
5		157.5, C		136.1, C	2.33, m,[b] α	48.3, CH	1.18 br m, α	48.3, CH
6	6.33, d (2.1), α	70.2, CH	6.10, d (2.1), α	71.1, CH	5.91, br s, α	70.6, CH	4.44, br s, α	69.2, CH
7	1.26, m, α	48.4, CH	1.11, m,[b] α	48.7, CH	1.21, m,[b] α	49.1, CH	0.88, m, α	49.5, CH
8	1.85, m[b]	19.9, CH_2	1.74, m[b]	20.5, CH_2	1.70, m[b], α	20.4, CH_2	1.63, m[b], α	20.4, CH_2
	1.85, m[b]		1.74, m[b]		1.63, qd (13.1, 3.5), β		1.52, qd (13.0, 3.0), β	
9	2.24, dt (13.1, 3.4), β	37.5, CH_2	2.10, dt (13.1, 3.0), β	38.4, CH_2	2.01, dt (13.1, 3.5), β	35.5, CH_2	1.94, dt (13.0, 3.0), β	39.3, CH_2
	1.38, td (13.1, 5.8), α		1.23, m, α		1.19, m[b], α		1.06, br t (13.0), α	
10		41.2, C		39.6, C		37.7, C		38.6, C
11	1.68, m	29.0, CH	1.59, m	29.1, CH	1.43, m	28.7, CH	1.61, m[b]	28.7, CH
12	1.00, d (5.2)	20.6, CH_3	0.95, d (6.7)	21.0, CH_3	0.86, d (6.7)	20.3, CH_3	0.97, d (7.5)	21.1, CH_3
13	0.98, d (5.2)	20.6, CH_3	0.94, d (6.7)	21.0, CH_3	1.01, d (6.7)	22.1, CH_3	0.96, d (7.5)	20.9, CH_3
14	1.32, s	16.4, CH_3	1.10, s	17.1, CH_3	1.07, br s	12.3, CH_3	1.37, br s	14.8, CH_3
15	4.63, d (12.5)	55.3, CH_2	2.04, s	17.4, CH_3	4.21, br d (12.0)	65.0, CH_2	4.30, d (11.3)	70.2, CH_2
	4.56, d (12.5)				3.95, br d (12.0)		4.13, d (11.3)	
1′		166.2, C		166.2, C		166.9, C		167.0, C
2′	6.40, d (16.0)	117.1, CH	6.40, d (15.9)	118.6, CH	6.39, d (15.9)	118.4, CH	6.45, d (16.2)	117.6, CH
3′	7.70, d (16.0)	146.1, CH	7.65, d (15.9)	144.8, CH	7.68, d (15.9)	145.2, CH	7.70, d (16.2)	145.7, CH
4′		134.0, C		134.5, C		134.4, C		134.3, C
5′, 9′	7.53, m	128.1, CH	7.51, m	128.2, CH	7.50, m	128.3, CH	7.53, m	128.3, CH
6′, 8′	7.39, m[b]	128.7, CH	7.36, m[b]	129.0, CH	7.36, m[b]	129.0, CH	7.38, m[b]	129.1, CH
7′	7.39, m[b]	130.5, CH	7.36, m[b]	130.4, CH	7.36, m[b]	130.5, CH	7.38, m[b]	130.7, CH

[a] ^{13}C extracted from $^{1}H-^{13}C$ 2D inverse-detected experiments. [b] Overlapping signals.

of **11** was established as 6β-cinnamoyloxy-1β,2α-dihydroxyeudesm-4(15)-ene.

Compound **12** was isolated as a colorless oil. The HRESIMS $[M + H]^+$ ion at m/z 369.2427 (calcd for $C_{24}H_{33}O_3^+$, 369.2424) established a molecular formula of $C_{24}H_{32}O_3$. Comprehensive analysis of its NMR data (Table 1) showed that **12** possesses a similar structure to those of **4**−**6**, with a double bond between C-4 and C-5. The only difference was at C-3, which appeared as a methylene (δ_H 2.17 and 2.09, δ_C 32.5), instead of an oxygen-bearing carbon. This was confirmed by a COSY correlation of H_2-2 (δ_H 1.66, overl.) with H_2-3 and by an HMBC correlation of H_3-15 (δ_H 1.88) with C-3 (δ_C 32.5) (Figure S39, Supporting Information). Thus, the structure of compound **12** was elucidated as 6β-cinnamoyloxy-1β-hydroxyeudesm-4-ene.

Compound **13** was obtained as a white solid. It showed a $[M + Na]^+$ adduct ion at m/z 421.1984 (calcd for $C_{24}H_{30}NaO_5^+$, 421.1986), corresponding to a molecular formula of $C_{24}H_{30}O_5$. By comparison of ^{1}H, ^{13}C, and 2D NMR data (Table 2) with those of the other isolated compounds, **13** proved to be structurally similar to **5**, with the only difference being that the methyl group at C-15 (**5**: δ_H 2.03, δ_C 11.0) was replaced by an oxygenated methylene (**13**: δ_H 4.63 and 4.56, δ_C 55.3). This assignment was corroborated by HMBC correlations of H_2-15 with C-3 (δ_C 198.5) and C-5 (δ_C 157.5) (Figure S46, Supporting Information). Hence, **13** was assigned as 6β-cinnamoyloxy-1β,15-dihydroxyeudesm-4-en-3-one.

Compound **14** was isolated as a yellow oil. Its molecular formula of $C_{24}H_{32}O_4$ was deduced from the HRESIMS $[M + H]^+$ ion at m/z 385.2361 (calcd for $C_{24}H_{33}O_4^+$, 385.2373) and from the ^{13}C NMR data. Comprehensive analysis of its NMR data (Table 2) indicated the same planar structure as for compound **6**. The relative configuration of both compounds was found to differ only in the orientation of the hydroxy group at C-3. For compound **6**, a J coupling constant for H-3/H-2 of 7.0 Hz corresponded to a dihedral angle of about 150° and suggested a β-orientation of the hydroxy group attached at C-3. This was also supported by the NOESY correlation of H-3 (δ_H 4.10, apparent q, J = 7.0 Hz) with H-2_{eq} (δ_H 2.11, ddd, J = 11.5/7.0/3.0 Hz) (Figure S7, Supporting Information). In compound **14**, the J coupling constant for H-3/H-2 of 4.0 Hz, arising from a dihedral angle of ca. 50°, indicated an alpha orientation of OH-3. This assignment was in agreement with the NOESY correlation of H-2_{ax} (δ_H 1.89) and H-3 (δ_H 4.06, br d, J = 4.0 Hz) (Figure S54, Supporting Information). Thus, the structure of **14** was established as 6β-cinnamoyloxy-1β,3α-dihydroxyeudesm-4-ene. Jakupovic et al.[28] previously reported H-3 (δ_H 4.08) in cinnamoyloxy-1β,3β-dihydroxyeudesm-4-ene (**6**) as a broad doublet with a coupling constant of 4 Hz. According to the NMR data of both epimers reported here, it seems likely that Jakupovic et al.[26] isolated in fact 6β-cinnamoyloxy-1β,3α-dihydroxyeudesm-4-ene and reported it erroneously as 6β-cinnamoyloxy-1β,3β-dihydroxyeudesm-4-ene.

Compound **15** was obtained as a yellow oil. Its HRESIMS showed an $[M + Na]^+$ adduct ion at m/z 407.2192 (calcd for $C_{24}H_{32}NaO_4^+$, 407.2193), corresponding to a molecular formula of $C_{24}H_{32}O_4$. Analysis of the NMR data (Table 2)

DOI: 10.1021/acs.jnatprod.7b00868
J. Nat. Prod. 2017, 80, 3296−3304

revealed a strong resemblance to **7**. The only difference was at C-15, which was an oxygenated methylene (**15**: δ_H 4.21 and 3.95, δ_C 65.0) instead of a methyl group (**7**: δ_H 1.70, δ_C 20.6). This was confirmed by HMBC correlations of H_{2a}-15 (δ_H 3.95) with C-3 (δ_C 124.4) and C-5 (δ_C 48.3) (Figure S62, Supporting Information). Thus, the structure of compound **15** was determined as 6*β*-cinnamoyloxy-1*β*,15-dihydroxyeudesm-3-ene.

Compound **16**, a yellow solid, gave a molecular formula of $C_{24}H_{34}O_5$ as established by the $[M + Na]^+$ ion at *m*/*z* 425.2295 (calcd for $C_{24}H_{34}NaO_5^+$, 425.2299) in the HRESIMS. It was thus an isomer of compound **9**. The ^{13}C NMR spectrum showed the same multiplicities as for **9**, suggesting the presence of a *trans*-decalin eudesmane skeleton with a cinnamoyloxy group. The ^1H and ^{13}C NMR data (Table 2) were fully assigned by 2D NMR ($^1H-^1H$ COSY, HSQC, and HMBC) experiments and revealed some differences between **9** and **16**. First, key HMBC correlations of H_2-15 (δ_H 4.30 and 4.13) to the carbonyl C-1′ (δ_C 167.0) inferred the attachment of the cinnamoyloxy group at H_2-15 (Figure 3). Consequently, H-6

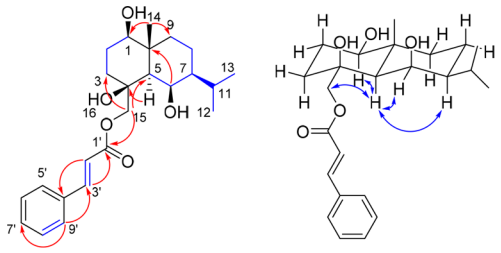


Figure 3. Key COSY (blue bonds), HMBC (red arrows), and NOESY correlations (blue arrows) of compound **16**.

was shifted upfield (δ_H 4.44, br s, δ_C 69.2) compared to the corresponding resonances in the cinnamoyl eudesmane derivatives **1**−**15**. Further differences from compound **9** were the presence of a hydroxy group at C-1 (δ_H 3.22, δ_C 79.7) and of a methylene at C-9 (δ_H 1.94 and 1.06, δ_C 39.3). The relative configuration of **16** was established as follows. Characteristic ^{13}C NMR shifts of C-14, C-10, and C-5[28] and 1,3-diaxial NOESY correlations indicated a *trans* junction of the decalin ring system (Figure 3). The *β*-equatorial orientation of the hydroxy group at C-1 was supported by the diaxial coupling H-1/H-2_{axial} (J = 12.0 Hz). The multiplicities of H-6 (δ_H 4.44, br s) and H-7 (δ_H 0.88, m) were similar to those found in compounds **1**−**15** and indicated their beta cofacial orientation. Finally, the configuration at C-4 was deduced from the NOESY contacts of H_2-15 and H-5. Compound **16** was thus assigned as 15-cinnamoyloxy-1*β*,4*β*,6*β*-trihydroxyeudesmane.

The absolute configuration of compound **12** was assigned by electronic circular dichroism (ECD). The experimental ECD spectrum of **12** showed a negative Cotton effect at 280 nm due to the $\pi \rightarrow \pi^*$ transition of the cinnamoyl group (Figure 4) and corresponding to a strong UV absorption maximum (Figure S40, Supporting Information). The experimental ECD spectrum of **12** matched well with the ECD curve calculated for the (1*R*,6*R*,7*S*,10*R*) enantiomer (Figure 4). The ECD spectra of compounds **4**−**6**, **13**, and **14** were similar to that of **12**. They all had the same allylic cinnamate group, and the ECD data suggested the same absolute configuration as for **12** (Figures S5, S6, S8, S47, and S55, Supporting Information). The absolute configuration of the remaining compounds was assigned tentatively based on biogenetic considerations and on the assumption that they possess the same absolute

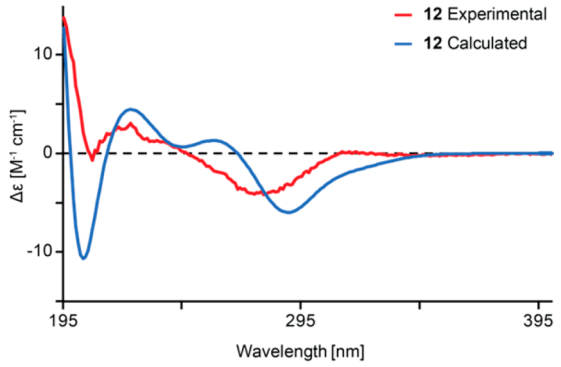


Figure 4. Experimental and calculated ECD spectra of compound **12** (1*R*,6*R*,7*S*,10*R*).

configuration at C-10. This was also in agreement with the reported absolute configuration of **2**, which had been established by X-ray single-crystal analysis.[29]

Antifungal Activity. The crude plant extract showed a minimal inhibitory concentration (MIC_{100}) against *P. viticola* of 35.4 *μ*g/mL, as determined in two independent experiments (Table 3). Owing to this promising activity in vitro, the extract

Table 3. In Vitro Minimal Inhibitory Concentrations (MIC_{100}) of *Verbesina lanata* Extract and Selected Constituents against *Plasmopara viticola*

	MIC_{100} [*μ*g/mL]			
compound	Exp 1[a]	Exp 2	Exp 3	mean[b]
2[c]	6.3	3.1	3.1	3.9
4	12.5	6.3	12.5	9.9
5	3.1	6.3	6.3	5.0
7	25	12.5	100	31.5
8	25	25	12.5	19.8
9	50	50	50	50.0
10[d]	6.3	6.3	12.5	7.9
14	12.5	6.3	12.5	9.9
16	25	50	50	39.7
extract		50	25	35.4

[a]Independent experiments. [b]Data log_2-transformed to calculate mean and retransformed to the linear scale. [c]**1**, **3**, **6**, **11**−**13**, and **15** were not tested due to the insufficient amounts available. [d]Tested sample had ca. 70% purity.

was then tested on grapevine seedlings. In a first attempt, however, no activity was detected, and the lack of activity was found to be due to insufficient solubility (data not shown). To overcome this issue, an emulsifiable concentrate formulation with 50 mg/g of extract was developed (VL-EC). At concentrations of 1 and 0.125 mg/mL of extract, the efficacies were 82% and 73%, respectively, as expressed as the lowering of infected leaf surface in the treated set of seedlings compared to the nontreated control set (disease severity of 92 ± 5%) (Figure 5).

Compounds **2**−**5**, **7**−**10**, **14**, and **16** were available in sufficient amounts to be tested against *P. viticola* in vitro (Table 3). Compounds **2**, **4**, **5**, **10**, and **14** exhibited MIC_{100} values of <10 *μ*g/mL and thus were significantly more active than the extract. These data confirmed that eudesmane sesquiterpenes are the antifungal constituents of *V. lanata* extract.

The genus *Verbesina* comprises a large number of species, but only few of these have been investigated phytochemically.

DOI: 10.1021/acs.jnatprod.7b00868
J. Nat. Prod. 2017, 80, 3296−3304

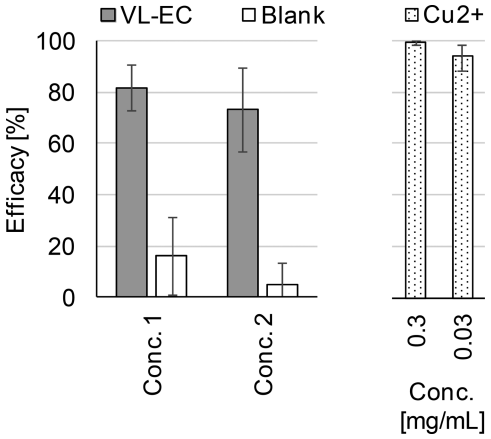


Figure 5. Efficacy of a formulated *Verbesina lanata* extract (VL-EC), blank formulation (Blank), and a copper control (Cu^{2+}) against *Plasmopara viticola* on grapevine seedlings under semicontrolled conditions. VL-EC contained 5% extract and 95% additives. VL-EC and the blank were tested at two concentrations ("Conc. 1": 20 mg/mL VL-EC (1 mg/mL of extract) or 19 mg/mL of formulation additives (blank); "Conc. 2": 2.5 mg/mL VL-EC [0.125 mg/mL of extract or 2.4 mg/mL of formulation additives (blank)]. The disease severity in the control was 92 ± 5%. The figure shows means and standard deviations of one experiment (n = 6).

Isoprenoids, in particular eudesmane sesquiterpenes, are reportedly the characteristic constituents of this genus.[23] Antifungal, antibacterial, and antiviral activity has been described for *Verbesina encelioides*, but the compounds responsible for the activity were not identified.[30] On the other hand, several eudesmane sesquiterpenes that have been isolated from other plants, mostly Asteraceae, have shown antifungal and antibacterial activity against human pathogens.[31−36] However, their efficacy against plant pathogens has not been previously reported. *V. lanata* ethyl acetate extract and five of the isolated compounds showed high inhibitory activity against *P. viticola*. The efficacy was comparable to that of other extracts, such as those from *Juncus effusus* (MIC_{100} 24 μg/mL; active constituent dehydroeffusol, MIC_{100} 4 μg/mL),[20] *Larix decidua* (MIC_{100} 23 μg/mL; active constituents larixyl acetate and larixol, MIC_{100} 6 and 14 μg/mL, respectively),[37] and *Inula viscosa*, *Yucca schidigera*, *Melaleuca alternifolia*, and *Quillaja saponaria* (all >80% efficacy in vivo at 1 mg/mL).[4] In contrast, extracts from *Achillea millefolium*, *Brassica napus*, *Glycyrrhiza glabra*, *Quercus* sp., *Salvia officinalis*, *Solidago virgaurea*, and *Rheum rhabarbarum* showed comparable activity to *V. lanata* at 10- to 15-fold higher concentration only.[4] In conclusion, *V. lanata* extracts and eudesmane sesquiterpenes could provide potential alternatives to copper fungicides, especially in organic farming. However, further studies under field conditions are required to substantiate their potential. The toxicological profiles of the plant extract and pure compounds also need to be investigated to assess the safety of such possible products.

EXPERIMENTAL SECTION

General Experimental Procedures. Formic acid, sulfuric acid, and solvents were obtained from Scharlau (Scharlab S. L.) or from Macron Fine Chemicals (Avantor Performance Materials). HPLC-grade solvents and ultrapure water from a Milli-Q water purification system (Merck Millipore) were used for HPLC. For extraction and preparative separation, technical grade solvents were used after distillation. $CDCl_3$ was purchased from Sigma-Aldrich.

Silica gel 60 F_{254} coated aluminum TLC plates and silica gel (0.063−0.200 mm) for open-column chromatography were obtained from Merck KGaA. TLC plates were visualized under UV light and by spraying with 1% vanillin (Roth GmbH + Co) in EtOH, followed by 10% sulfuric acid in EtOH and heating at 110 °C.

HPLC-PDA-ESIMS analyses were performed on an LC-MS 8030 system (Shimadzu) using a SunFire C_{18} (3.5 μm, 150 × 3.0 mm i.d.) column equipped with a guard column (10 mm × 3.0 mm i.d.) (Waters). LabSolutions software (Shimadzu) was used for data acquisition and processing.

Semipreparative HPLC was performed on an Agilent 1100 Series instrument with a PDA detector. A SunFire C_{18} (5 μm, 150 × 10 mm i.d.) column with a guard column (10 mm × 10 mm i.d.) (Waters) or a Nucleodur CN NP (5 μm, 150 × 10 mm i.d.) column with a guard column (10 mm × 8 mm i.d.) (Macherey-Nagel) was used. Data acquisition and processing was performed using ChemStation software (Agilent Technologies).

Preparative HPLC was carried out on a Puriflash 4100 system (Interchim) or a Reveleris PREP purification system (Büchi). A SunFire C_{18} (5 μm, 150 × 30 mm i.d.) column with guard column (10 mm × 20 mm i.d.) (Waters) was used for separations.

HRESIMS data were recorded in positive ion mode on an Agilent 1290 Infinity system with an Agilent 6540 UHD Accurate-Mass quadrupole time-of-flight detector. Optical rotations were measured in MeOH on a P-2000 digital polarimeter (Jasco) equipped with a sodium lamp (589 nm) and a 10 cm temperature-controlled microcell. UV and ECD spectra were recorded, at a concentration of 0.2 mg/mL in MeOH, on a Chirascan CD spectrometer with 1 mm path precision cells 110 QS (Hellma Analytics). NMR spectra were recorded on a Bruker AVANCE III 500 MHz spectrometer operating at 500 MHz for ^1H and 125 MHz for ^{13}C. The instrument was equipped with a 1 mm TXI microprobe operated at 18 °C or a 5 mm BBO probe at 23 °C (Bruker Biospin). Chemical shifts are reported as δ values (ppm), with residual solvent signal as internal reference, J in Hz. Standard pulse sequences from the Topspin 2.1 software package were used.

Plant Material. Inflorescences of *Verbesina lanata* were collected in November 2001 in Campana, Panama, by CIFLORPAN (Center for Pharmacognostic Research on Panamanian Flora), Panamanian collection number FLORPAN 5456. A voucher specimen is deposited at the Herbarium of the University of Panama. The taxonomic identity was confirmed by Alex Espinosa, botanist at CIFLORPAN. The material was air-dried and minced in Panama. A voucher specimen (no. 948) is also available at the Division of Pharmaceutical Biology, Department of Pharmaceutical Sciences, University of Basel.

Extraction and Isolation. Powdered inflorescences (300 g) were mixed with sea sand and percolated sequentially at room temperature with petroleum ether (3.5 L), ethyl acetate (8.6 L), and methanol (6.5 L) to afford, after evaporation under reduced pressure, 8.8 g of petroleum ether extract, 7.6 g of ethyl acetate extract, and 22.8 g of methanol extract. A portion of the ethyl acetate extract (6.8 g) was dissolved in a mixture of dichloromethane and ethyl acetate and adsorbed onto ca. 20 g of silica gel. The dried powder was then loaded onto an open column filled with silica gel (65 cm × 5 cm i.d.). Elution was performed with a step gradient of n-hexane/ethyl acetate [95:5 (2.5 L), 90:10 (2.0 L), 85:15 (1.0 L), 80:20 (3.0 L), 70:30 (2.0 L), 60:40 (2.0 L), 50:50 (2.0 L), 30:70 (2.0 L), and 0:100 (2.5 L)], followed by ethyl acetate/methanol [95:5 (2.0 L), and 80:20 (2.0 L)], at a flow rate of approximately 25 mL/min. A total of 26 fractions (Frs A−Z) were collected based on TLC analysis.

Fraction P (28 mg) was submitted to semipreparative RP-HPLC with a gradient of 40% to 100% acetonitrile in 30 min at a flow rate of 4 mL/min. Repeated injections afforded compound **1** (1.0 mg, t_R 27.8 min). With the aid of preparative RP-HPLC (Puriflash system) compounds **3** (4.0 mg, t_R 18.3 min), **4** (8.5 mg, t_R 19.6 min), **7** (34.9 mg, t_R 26.8 min), and **12** (1.5 mg, t_R 28.3 min) were isolated from a portion (110 mg) of Fr Q (122 mg). Separation was achieved with a gradient of 50% to 100% acetonitrile in 30 min at a flow rate of 20 mL/min. Compound **5** (43.7 mg, t_R 19.2 min) was obtained by preparative RP-HPLC (Reveleris PREP purification system) of a portion (200 mg) of Fr U (500 mg). Separation was achieved with a

DOI: 10.1021/acs.jnatprod.7b00868
J. Nat. Prod. 2017, 80, 3296−3304

gradient of 50% to 100% acetonitrile in 30 min at a flow rate of 25 mL/min. Compounds **2** (41.7 mg, t_R 28.6 min), **8** (10.1 mg, t_R 23.9 min), and **16** (11.2 mg, t_R 21.6 min) were obtained from a portion (300 mg) of Fr W (304 mg). Separation was achieved by preparative RP-HPLC (Puriflash system), using a gradient of 30% to 100% acetonitrile in 30 min; the flow rate was 20 mL/min. A portion (542 mg) of Fr X (1325 mg) was separated by preparative RP-HPLC (Puriflash system) into 10 subfractions (Frs X1−X10) with 50% acetonitrile for 45 min and a flow rate of 20 mL/min. Subfractions X2, X5, and X7 afforded compounds **9** (6.6 mg, t_R 16.0 min), **15** (8.0 mg, t_R 24.0 min), and **14** (30.6 mg, t_R 29.6 min), respectively. Further purification of subfractions X4, X9, and X10 by semipreparative RP-HPLC afforded compounds **13** (2.2 mg, t_R 37.0 min), **6** (1.1 mg, t_R 48.3 min), and **11** (9.5 mg, t_R 25.5 min), respectively. Separations were performed with 65% B (X4) and 68% (X9) and 73% (X10) methanol at a flow rate of 4 mL/min. About half of Fr X6 (27 mg) was further purified by semipreparative CN NP-HPLC with *n*-heptane/2-propanol (97:3) at a flow rate of 3 mL/min to afford **10** (9.0 mg, t_R 16.0 min). The purity of compounds, as assessed by ^{1}H NMR spectroscopy, was >95% for **2**−**6**, **8**, **10**, **11**, **13**, and **16**; >90% for **1**, **7**, **9**, **12**, and **14**; and >80% for **15**, which contained ca. 20% of another, unidentified, eudesmane derivative.

Microfractionation of Fractions for Activity Profiling. Microfractionation of the active fractions (Frs P, Q, U, W, and X) was carried out by analytical RP-HPLC on an LC-MS 8030 system (Shimadzu) connected with an FC 204 fraction collector (Gilson). For each fraction, a solution of 10 mg/mL was prepared in DMSO. Altogether four injections were performed: 3 × 30 μL with only UV detection (254 nm) for collection (0.9 mg of fraction in total) and 1 × 10 μL with UV-ESIMS detection without collection. The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The flow rate was 0.4 mL/min. Gradients applied were as follows: Frs P and Q, 40% to 100% B in 30 min; Fr. U, 40% to 100% B in 40 min; Fr. W, 30% to 100% B in 30 min; Fr. X, 30% to 70% B in 40 min. In each case, the column was washed with 100% B for 5 min. Fractions of 1 min each were collected from minute 2 to the end of the method (Frs P, Q, and W: 33 fractions; U and X: 43 fractions). Microfractions from the three successive injections of a given sample were collected into the corresponding wells of a 96-deep-well plate. Plates were then dried in a Genevac EZ-2 evaporator.

HPLC-PDA-ESIMS Analysis. Analyses were performed on an LC-MS 8030 system (Shimadzu) using a SunFire C_{18} (3.5 μm, 150 × 3.0 mm i.d.) column equipped with a guard column (10 mm × 3.0 mm i.d.) (Waters). The software for data acquisition and processing was LabSolutions (Shimadzu). UV and mass detection ranges were 190 to 600 nm and *m*/*z* 160−1500, respectively. The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). A gradient of 30% to 100% B in 30 min was applied, followed by 100% B for 5 min. The flow rate was 0.4 mL/min. The samples were dissolved in DMSO (extract 10 mg/mL, fractions 5 mg/mL), and 10 μL was injected. Compounds were identified in the extract or fractions by comparison of their ESIMS data and retention times with those of the purified compounds.

6β-Cinnamoyloxy-4β-hydroxyeudesmane (**1**): colorless oil; $[\alpha]^{25}_D$ −12 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.93), 211 (3.86), 217 (3.91), 222 (3.86), 278 (4.00) nm; ^{1}H and ^{13}C NMR, Table S1, Supporting Information; HRESIMS *m*/*z* 393.2399 [M + Na]$^+$ (calcd for $C_{24}H_{34}NaO_3^+$, 393.2400).

6β-Cinnamoyloxy-3β,4α-dihydroxyeudesmane (**2**): pale yellow plates (ethyl acetate); $[\alpha]^{25}_D$ −59 (*c* 0.1, MeOH), UV (MeOH) λ_{max} (log ε) 204 (4.19), 217 (4.23), 222 (4.17), 279 (4.41) nm; ^{1}H and ^{13}C NMR, Table S1, Supporting Information; HRESIMS *m*/*z* 409.2347 [M + Na]$^+$ (calcd for $C_{24}H_{34}NaO_4^+$, 409.2349).

6β-Cinnamoyloxy-3α-hydroperoxy-1β-hydroxyeudesm-4(15)-ene (**3**): colorless oil; $[\alpha]^{25}_D$ 20 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.19), 217 (4.18), 222 (4.12), 279 (4.32) nm; ^{1}H and ^{13}C NMR, Table S1, Supporting Information; HRESIMS *m*/*z* 423.2130 [M + Na]$^+$ (calcd for $C_{24}H_{32}NaO_5^+$, 423.2142).

6β-Cinnamoyloxy-3α-hydroperoxy-1β-hydroxyeudesm-4-ene (**4**): colorless oil; $[\alpha]^{25}_D$ −47 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.35), 217 (4.28), 222 (4.20), 278 (4.36) nm; ^{1}H and ^{13}C NMR, Table S1, Supporting Information; HRESIMS *m*/*z* 423.2134 [M + Na]$^+$ (calcd for $C_{24}H_{32}NaO_5^+$, 423.2142).

6β-Cinnamoyloxy-1β-hydroxyeudesm-4-en-3-one (**5**): colorless oil; $[\alpha]^{25}_D$ −94 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.10), 217 (4.16), 223 (4.15), 255 (sh) (4.14), 279 (4.29) nm; ^{1}H and ^{13}C NMR, Table S2, Supporting Information; HRESIMS *m*/*z* 405.2035 [M + Na]$^+$ (calcd for $C_{24}H_{30}NaO_4^+$, 405.2036).

6β-Cinnamoyloxy-1β,3β-dihydroxyeudesm-4-ene (**6**): white, amorphous solid; $[\alpha]^{25}_D$ −80 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.25), 216 (4.15), 222 (4.04), 277 (4.25) nm; ^{1}H and ^{13}C NMR, Table S2, Supporting Information; HRESIMS *m*/*z* 407.2191 [M + Na]$^+$ (calcd for $C_{24}H_{32}NaO_4^+$, 407.2193).

6β-Cinnamoyloxy-1β-hydroxyeudesm-3-ene (**7**): colorless oil; $[\alpha]^{25}_D$ 34 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.23), 217 (4.20), 222 (4.12), 277 (4.38) nm; ^{1}H and ^{13}C NMR, Table S2, Supporting Information; HRESIMS *m*/*z* 369.2425 [M + H]$^+$ (calcd for $C_{24}H_{33}O_3^+$, 369.2424).

7-epi-6α-Cinnamoyloxy-1β,4α-dihydroxyeudesmane (**8**): pale yellow oil; $[\alpha]^{25}_D$ −13 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.10), 217 (4.11), 223 (4.06), 280 (4.27) nm; ^{1}H and ^{13}C NMR, see Table S2, Supporting Information; HRESIMS *m*/*z* 387.2510 [M + H]$^+$ (calcd for $C_{24}H_{35}O_4^+$, 387.2530).

6β-Cinnamoyloxy-4β,9β,15-trihydroxyeudesmane (**9**): pale yellow oil; $[\alpha]^{25}_D$ 2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.06), 210 (4.00), 216 (4.04), 222 (3.98), 276 (4.15) nm; ^{1}H and ^{13}C NMR, Table 1; HRESIMS *m*/*z* 425.2297 [M + Na]$^+$ (calcd for $C_{24}H_{34}NaO_5^+$, 425.2299).

6β-Cinnamoyloxy-1β,3α-dihydroxyeudesm-4(15)-ene (**10**): pale yellow, amorphous solid; $[\alpha]^{25}_D$ 29 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.11), 217 (4.12), 223 (4.06), 279 (4.28) nm; ^{1}H and ^{13}C NMR, Table 1; HRESIMS *m*/*z* 407.2192 [M + Na]$^+$ (calcd for $C_{24}H_{32}NaO_4^+$, 407.2193).

6β-Cinnamoyloxy-1β,2α-dihydroxyeudesm-4(15)-ene (**11**): yellow oil; $[\alpha]^{25}_D$ 76 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.15), 217 (4.14), 223 (4.08), 278 (4.30) nm; ^{1}H and ^{13}C NMR, Table 1; HRESIMS *m*/*z* 385.2373 [M + H]$^+$ (calcd for $C_{24}H_{33}O_4^+$, 385.2373).

6β-Cinnamoyloxy-1β-hydroxyeudesm-4-ene (**12**): colorless oil; $[\alpha]^{25}_D$ 1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.20), 216 (4.11), 222 (4.02), 278 (4.22) nm; ECD (MeOH, $c = 5.4 \times 10^{-4}$ M, 1 mm path length) λ_{max} (Δε) 207 (−0.68), 223 (+3.08), 231 (+1.22), 237 (+1.23), 279 (−4.18), 312 (+0.16) nm; ^{1}H and ^{13}C NMR, Table 1; HRESIMS *m*/*z* 369.2427 [M + H]$^+$ (calcd for $C_{24}H_{33}O_3^+$, 369.2424).

6β-Cinnamoyloxy-1β,15-dihydroxyeudesm-4-en-3-one (**13**): white solid; $[\alpha]^{25}_D$ −115 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.17), 218 (4.23), 223 (4.22), 255 (sh) (4.10), 280 (4.42) nm; ^{1}H and ^{13}C NMR, Table 2; HRESIMS *m*/*z* 421.1984 [M + Na]$^+$ (calcd for $C_{24}H_{30}NaO_5^+$, 421.1986).

6β-Cinnamoyloxy-1β,3α-dihydroxyeudesm-4-ene (**14**): yellow oil; $[\alpha]^{25}_D$ −23 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.19), 217 (4.07), 222 (4.00), 277 (4.21) nm; ^{1}H and ^{13}C NMR, Table 2; HRESIMS *m*/*z* 385.2361 [M + H]$^+$ (calcd for $C_{24}H_{33}O_4^+$, 385.2373).

6β-Cinnamoyloxy-1β,15-dihydroxyeudesm-3-ene (**15**): yellow oil; $[\alpha]^{25}_D$ 14 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 203 (sh) (4.14), 217 (4.10), 223 (4.03), 279 (4.21) nm; ^{1}H and ^{13}C NMR, Table 2; HRESIMS *m*/*z* 407.2192 [M + Na]$^+$ (calcd for $C_{24}H_{32}NaO_4^+$, 407.2193).

15-Cinnamoyloxy-1β,4β,6β-trihydroxyeudesmane (**16**): yellow solid; $[\alpha]^{25}_D$ −12 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.15), 217 (4.17), 222 (4.11), 278 (4.31) nm; ^{1}H and ^{13}C NMR, Table 2; HRESIMS *m*/*z* 425.2295 [M + Na]$^+$ (calcd for $C_{24}H_{34}NaO_5^+$, 423.2299).

Computational Methods. Conformational analysis of compound **12** was performed with MacroModel 9.8 software (Schrödinger LLC) employing the OPLS 2005 (Optimized Potential for Liquid Simulations) force field in H_2O. Conformers within a 2 kcal/mol energy window from the global minimum were selected for geometrical optimization and energy calculation using density function theory (DFT) with Becke's nonlocal three-parameter exchange and

DOI: 10.1021/acs.jnatprod.7b00868
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correlation functional and the Lee−Yang−Parr correlation functional level (B3LYP) using the B3LYP/6-31G** basis set in the gas phase with the Gaussian 09 program package.[38] Vibrational evaluation was done at the same level to confirm minima. Excitation energy (denoted by wavelength in nm), rotatory strength dipole velocity (R_{vel}), and dipole length (R_{len}) were calculated in MeOH by TD-DFT/B3LYP/6-31G**, using the SCRF method, with the CPCM model. ECD curves were obtained on the basis of rotatory strengths with a half-band of 0.24 eV and UV shift using SpecDis v1.64.[39] ECD spectra were calculated from the spectra of individual conformers according to their contribution calculated by Boltzmann weighting.

In Vitro Antifungal Bioassays. Fractions A−Z (26 fractions) obtained from open column chromatography were dissolved at a concentration of 5 mg/mL in DMSO. A 7.5 μL amount of the stock solutions was added to 96-well plates containing 117.5 μL of mineral water and were then serially diluted in the test plate 1:5 and 1:25 with mineral water. Next, 20 μL of a continuously stirred sporangia suspension of *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni ((1.8−2.5) × 10^5 sporangia/mL) (prepared as described below in the subsection "In Vivo Assays on Seedlings") was added to each well. The resulting test concentrations were 250, 50, and 10 μg/mL, respectively.

For the determination of MIC_{100}, defined as the concentration needed to completely inhibit the activity of zoospores, the crude extract and pure compounds **2**, **4**, **5**, **7**−**10**, **14**, and **16** were dissolved in DMSO at a concentration of 2 mg/mL. Compounds **1**, **3**, **6**, **11**−**13**, and **15** were not tested due to the insufficient amounts available. Each solution was then serially diluted 1:1 in 50% DMSO to 3.9 μg/mL. Aliquots of 6 μL of each concentration were added to 94 μL of mineral water before adding 20 μL of the sporangia solution ((1.8−2.5) × 10^5 sporangia/mL). Resulting concentrations of the test products were between 0.195 and 100 μg/mL.

In all in vitro experimental sets, the effect of the solvent (DMSO) alone was tested in at least two replicates in all relevant concentrations.

The activity of zoospores was assessed 2−3 h after setup of the experiment using a binocular at magnifications of 50- to 100-fold. For determination of MIC_{100}, a distinction was made between "no zoospores germinated, or all zoospores inactive" and "active zoospores present". For assessment of activity of fractions and microfractions, inhibition levels were scored as follows: 0, similar to water control; 1, distinct reduction in number and/or activity of zoospores; 2, no zoospores germinated, or all zoospores inactive. To visualize overall inhibitory activity, inhibition levels of all three concentrations were summed up, resulting in values between 0 (no inhibition at highest tested concentrations) and 6 (complete inhibition down to lowest tested concentration).

To calculate mean MIC_{100}, data were $\log_2$ transformed. Data were then retransformed to linear scale.

In Vivo Assays on Seedlings. Bioassays were carried out under semicontrolled conditions in experimental facilities (greenhouse and growth chambers). Small grapevine (*Vitis vinifera* L.) cv. "Chasselas" seedlings were transplanted to individual pots (0.275 L) containing a standard substrate ("Einheitserde Typ 0", Gebr. Patzer GmbH & Co. KG) previously amended with 3 g/L of a mineral fertilizer (Tardit 3M, Hauert Günther Düngerwerke GmbH). Plants were grown in the greenhouse at a minimal temperature of 18 °C under natural light. The photoperiod was extended with sodium high-pressure lamps to 16 h. Plants were used for bioassays when they had three or four fully developed leaves (2−3 weeks after transplanting).

Each experimental set included a nontreated noninoculated control, a water-treated inoculated control, and a standard treatment (copper hydroxide, Kocide Opti, DuPont de Nemours) at two concentrations (300 and 30 μg/mL of copper). All experiments included six replicate plants per treatment. A formulation of *V. lanata* extract was used, containing 5% of the extract, 84% of a solvent (ethyl acetate), and 11% of an emulsifier (Emulsogen EL360, Clariant) in order to enhance solubility in water. The formulation was added to demineralized water at concentrations of 1, 0.5, 0.25, and 0.125 mg plant extract/mL. As a control, a blank formulation was tested at corresponding concentrations. Plants were sprayed with the test products using an air-assisted hand sprayer (DeVilbiss Compact MINI HVLP Touch-Up spray gun) until the leaves (adaxial and abaxial side) were completely covered with a dense layer of small droplets. Plants were left subsequently to dry at room temperature before inoculation. *P. viticola* sporangia suspensions were prepared from previously infected plants by washing freshly sporulating grapevine leaves with water and filtering through cheese cloth. The concentration of the sporangia suspensions was adjusted to 5 × 10^4 sporangia/mL. Plants were spray-inoculated using the air-assisted hand sprayer on the abaxial leaf side. Inoculated plants were subsequently incubated at 20−21 °C and 80−99% relative humidity (RH) in the light for 24 h. Then, plants were maintained at 20 °C, 60−80% RH, with a 16/8-h day/night light regime. Next, 5 to 6 days after inoculation, plants were incubated overnight in the dark at 20 °C and 80−99% RH to promote sporulation. Disease incidence (the percentage of leaves with disease symptoms) and disease severity (the percentage of leaf area covered by lesions) were assessed 6 to 7 days after inoculation. All disease assessments were made using continuous values of percentage based on the European and Mediterranean Plant Protection Organization (EPPO) standard scale.[40]

Efficacies were calculated according to Abbott[41] as $(1 - (A \times B^{-1})) \times 100$, with A = disease severity on an individual plant and B = mean disease severity of control plants.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00868.

HPLC-activity profiling of active fractions (Frs P, Q, U, W, and X); ^{1}H and ^{13}C NMR spectroscopic data for compounds **1**−**8**; ECD spectra of compounds **1**−**16**; 1D selective NOESY of compound **6**; key COSY, HMBC, and NOESY correlations of compounds **10**−**15**; UV spectrum of compound **12** in MeOH; and 1D and 2D NMR spectra of compounds **9**−**16** (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank T. Hettich (Group of Prof. Dr. G. Schlotterbeck, School of Life Sciences, University of Applied Sciences, Institute for Chemistry and Bioanalytics, Muttenz) for recording the HRESIMS data and Alex Espinosa, botanist of CIFLORPAN, for the identification and the picture of *V. lanata* (abstract graphic). Financial support by Coop Sustainability Fund is gratefully acknowledged. M.P.G. wishes to thank the SNI program of SENACYT Panama for support.

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