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Fungicidal Sesquiterpene Dialdehyde Cinnamates from *Pseudowintera axillaris*

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Bioactivity-directed separation of a foliage extract from the New Zealand shrub *Pseudowintera axillaris* led to a compound with fungicidal activity against the plant pathogen *Phytophthora infestans*. This was identified as a new sesquiterpene dialdehyde cinnamate named paxidal. Two 6-hydroxy derivatives were present at lower levels in the extract. A further nine derivatives were synthesized from these natural products for a structure−activity study against a range of important food crop pathogens. The cinnamate group was important for fungicidal effects, and protection of the dialdehyde as a dimethyl acetal gave more potent, broader spectrum activity.

KEYWORDS: ***Pseudowintera*; sesquiterpene; dialdehyde; cinnamate; antifungal activity; *Phytophthora***

INTRODUCTION

Modern agriculture relies on effective control of fungal diseases to increase crop yield and quality and consequently increase crop value (*1*). No single fungicide can be used for all disease situations, and the widespread use of fungicides can select for fungicide-resistant pathogens (*2*). Therefore, there is a need for safer and more cost-effective fungicides which are easier to use and provide better performance against resistant pathogens. During screening of New Zealand plants for biologically active natural products (*3*), an extract from foliage of *Pseudowintera axillaris* showed in vivo antifungal activity against the plant pathogen *Phytophthora infestans* (PHYTIN). *P. axillaris* (J. R. et G. Forst.) Dandy (family Winteraceae) is a shrub found in forests throughout the North Island and the northwestern South Island of New Zealand (*4*). It is one of three species in this endemic genus (*5*). The more common *P. colorata* contains polygodial (**1**) and 9-deoxymuzigadial (**2**) (*6, 7*), which have antifungal activity, in particular against the human pathogen *Candida albicans* (*8*). However, an extract of *P. axillaris* had no activity against *C. albicans* and did not contain any detectable polygodial (*7*). There are no other published studies of the chemistry of *P. axillaris*.

We now report the bioactivity-directed isolation of a new sesquiterpene dialdehyde cinnamate **3** as the fungicidal compound in *P. axillaris*. Two 6-hydroxy-derivatives **4** and **5** were present at lower levels in the extract. A further nine derivatives **6**−**14** were synthesized from these natural products for a structure−activity study against important food crop pathogens.

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Table 1. ^{13}C NMR Data for Paxidal (**3**) and Related Compounds **4**–**14**[a]

atom	3	4	5	6	7	8	9	10	11	12	13	14
1	80.5	81.0	79.8	78.8	87.7	79.3	78.9	79.0	78.1	78.8	78.2	82.1
2	36.0	35.7	36.4	40.0	34.5	36.8	37.0	36.7	38.7	37.3	38.7	37.2
3	35.6	35.5	36.0	35.8	35.4	35.5	36.0	35.9	36.3	36.6	36.1	35.9
4	149.3	146.4	147.0	149.8	150.2	150.4	147.5	147.3	148.0	156.9	147.6	151.1
5	44.8	49.2	53.9	44.3	44.9	44.5	54.7	54.6	54.1	57.8	50.7	45.5
6	25.9	64.6	64.7	26.1	26.0	24.6	65.6	65.3	65.3	194.8	73.8	24.4
7	150.8	148.0	150.4	151.7	150.9	119.9	121.4	121.8	122.8	121.8	118.8	126.8
8	140.3	140.3	139.1	139.5	140.7	136.8	139.3	138.9	137.5	142.7	138.6	137.2
9	56.7	56.8	56.2	57.3	57.6	54.6	54.5	53.8	53.8	54.9	53.9	51.2
10	42.9	42.1	44.1	43.8	44.3	39.0	42.8	42.6	43.7	45.1	43.6	41.0
11	200.4	199.8	199.7	203.4	200.7	106.9	107.0	106.6	106.5	107.1	106.6	62.7
12	192.5	193.1	192.3	193.0	192.8	103.6	103.4	103.4	103.3	102.9	103.4	66.9
13	107.9	110.5	108.4	107.3	107.1	106.3	107.3	107.3	107.1	111.1	107.1	106.4
14	17.9	17.9	17.6	17.9	18.1	15.0	17.8	17.7	18.0	17.5	18.1	17.8
15	10.6	13.7	10.9	8.2	9.3	7.6	8.7	8.5	7.9	9.5	7.9	9.0
1′	166.1	166.1	165.9			165.5	165.6	171.7		165.5		166.6
2′	117.7	117.4	117.3			118.7	118.6	30.8		118.1		118.2
3′	146.1	146.1	146.3			143.8	144.4	36.0		144.9		145.4
4′	134.1	134.0	134.0			134.3	134.3	140.4		134.1		134.2
5′+9′	128.9	128.9	128.9			128.7	129.0	128.5		129.0		128.2
6′+8′	128.4	128.3	128.3			127.7	128.0	128.2		128.0		128.9
7′	130.6	130.7	130.7			130.0	130.3	126.2		130.5		130.5
11OMe						56.0	56.4	55.2	56.2	56.5	56.2	
12OMe						53.8	54.3	54.4	56.4	54.7	54.9	
1/ 6OMe					56.5						54.4	

[a] In $CDCl_3$, chemical shift in ppm.

MATERIALS AND METHODS

General Experimental Procedures. All solvents were distilled before use and were removed by rotary evaporation at temperatures up to 35 °C. Octadecyl-functionalized silica gel (C18) was used for reversed-phase (RP) flash chromatography, and Merck silica gel 60, 200−400 mesh, 40−63 μm, was used for silica gel flash chromatography. TLC was carried out using Merck DC-plastikfolien Kieselgel

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Table 2. ^{1}H NMR Data for Paxidal (**3**) and Related Compounds **4**–**14**[a]

atom	3	4	5	6	7	8	9	10	11	12	13	14
1	5.14 (dd,1 2,4)	5.05 (dd,1 1.5,4)	5.12 (dd,1 1.5,4. 5)	3.86 (dd,1 2,4)	3.26 (dd,1 2,4)	4.97 (dd,1 2,4)	5.04 (dd,11 .5,4.5)	4.86 (dd,1 3.5)	3.67 (dd,1 2.5)	5.15 (dd,1 2.5,5)	3.71 (ddd, 13,4.3)	5.14 (dd,1 2,4.5)
2ax	1.26 (q,12)	1.40 (q,12)	1.27 (q,12)	1.23 (q,12)	1.06 (q,12)	1.25 (q,12. 5)	1.50 (q,12)	1.02 (m)	1.10 (q,12)	1.22 (m)	1.19 (q,14)	1.35 (q,12. 5)
2eq	2.08 (dt,1 2,4)	2.04 (dt,1 2.5,4)	2.12 (dt,1 2,4)	1.85 (dt,1 2.5,4)	2.06 (m)	1.95 (dt,12 ,4)	2.02 (dt,12. 5,4)	2.12 (dt,14 .4.5)	1.80 (dt,1 2,5)	2.07 (dt,12 ,5)	1.81 (dt,14 ,4)	1.91 (dt,12 .5,4.5)
3	2.28 (m)	2.31 (m)	2.26 (m)	2.13 (m)	2.06 (m)	2.23 (m)	2.24 (m)	2.15 (m)	2.05 (m)	2.19 (m)	2.11 (m)	2.22 (m)
5	2.21 (m)	2.20 (s)	2.19 (d,9. 5)	2.06 (dd,1 1,4)	2.06 (m)	2.20 (m)	2.18 (d,9)	2.12 (d,10)	2.00 (d,10)	2.87 (s)	2.15 (d,10)	2.15 (m)
6ax	2.46 (m)	OH 2.04 (bs)	4.76 (dt,9. 5,2)	2.43 (m)	2.48 (m)	2.16 (m)	4.55 (dt,9, 3)	4.50 (dt,10 ,3.5)	4.48 (dt,1 0,3)		4.07 (dt,10 ,3)	2.10 (m)
6eq	2.46 (m)	4.81 (m)	OH 1.63 (bs)	2.5 (m)	2.39 (m)	2.16 (m)	OH 1.61 (bs)					2.06 (m)
7	7.07 (bm)	7.10 (dd,6 ,2)	6.94 (bs)	7.11 (bm)	7.03 (bm)	5.78 (q,3)	5.85 (t,3)	5.83 (t,3)	5.84 (t,3)	6.05 (d,3)	6.01 (t,3)	5.89 (bd,6)
9	3.52 (bs)	3.54 (bs)	3.56 (q,2)	3.42 (bs)	3.40 (bs)	2.65 (bt,2)	2.82 (q,3)	2.76 (q,3.5)	2.7 (m)	3.08 (m)	2.75 (m)	2.49 (bm)
11	9.89 (d,2. 5)	9.87 (d,2)	9.86 (d,2)	9.85 (d,2)	9.93 (d,2)	5.03 (d,4)	5.08 (d,4)	5.05 (d,4)	4.99 (d,5)	5.27 (d,3)	5.01 (d,6)	4.18 (dd,1 1,2.5), 3.76 (dd,1 1,4.5)
12	9.36 (s)	9.44 (s)	9.43 (s)	9.42 (s)	9.38 (s)	5.05 (s)	5.14 (s)	5.11 (s)	5.13 (s)	5.30 (s)	5.16 (s)	4.27 (d,12), 4.00 (d,12)
13E	5.00 (s)	5.17 (s)	5.21 (s)	4.95 (s)	4.94 (s)	4.88 (s)	5.11 (bs)	5.07 (bs)	5.06 (s)	5.20 (bs)	5.03 (t,1.5)	4.88 (s)
13Z	4.84 (s)	5.60 (s)	5.07 (s)	4.78 (s)	4.77 (s)	4.76 (s)	5.07 (bs)	5.04 (bs)	5.02 (s)	5.37 (bs)	4.85 (bs)	4.72 (s)
14	1.13 (d,6. 5)	1.16 (d,7)	1.15 (d,7)	1.10 (d,6.5)	1.14 (d,6)	1.06 (d,6.5)	1.12 (d,6.5)	1.07 (d,7)	1.10 (d,7)	1.10 (d,6.5)	1.12 (d,7)	1.09 (d,6.5)
15	0.84 (s)	1.12 (s)	0.88 (s)	0.66 (s)	0.63 (s)	0.74 (s)	0.87 (s)	0.75 (s)	0.68 (s)	0.99 (s)	0.69 (s)	0.87 (s)
2′	6.41 (d,16)	6.42 (d,16)	6.39 (d,16)			6.39 (d,16)	6.40 (d,16)	2.62 (m)		6.41 (d,16)		6.47 (d,16)
3′	7.66 (d,16)	7.69 (d,16)	7.68 (d,16)			7.62 (d,16)	7.66 (d,16)	2.97 (t,8)		7.69 (d,16)		7.71 (d,16)
5′+9′	7.52 (m)	7.53 (m)	7.53 (m)			7.50 (m)	7.52 (m)	7.25 (m)		7.53 (m)		7.53 (m)
6′+8′	7.38 (m)	7.39 (m)	7.39 (m)			7.35 (m)	7.38 (m)	7.18 (m)		7.38 (m)		7.38 (m)
7′	7.38 (m)	7.39 (m)	7.39 (m)			7.35 (m)	7.38 (m)	7.18 (m)		7.38 (m)		7.38 (m)
11O Me						3.18 (s)	3.23 (s)	3.38 (s)	3.49 (s)	3.27 (s)	3.51 (s)	
12O Me						3.30 (s)	3.37 (s)	3.34 (s)	3.39 (s)	3.40 (s)	3.36 (s)	
1/ 6OM e					3.41 (s)						3.42 (s)	

[a] In $CDCl_3$, chemical shift in ppm (multiplicity, coupling constants in Hz).

60 F_{254}, first visualized with a UV lamp, and then by dipping in a vanillin solution (1% vanillin, 1% H_2SO_4 in EtOH), and heating. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Mass, UV, and IR spectra were recorded on Kratos MS-80, Shimadzu UV 240, and Perkin-Elmer 1600 FTIR instruments, respectively. NMR spectra of $CDCl_3$ solutions at 25 °C were recorded at 500 or 300 MHz for ^{1}H and at 125 or 75 MHz for ^{13}C on Varian INOVA-500 or -300 spectrometers. Chemical shifts are given in ppm on the δ-scale referenced to solvent peaks: $CHCl_3$ at 7.25 and $\mathbf{C}DCl_3$ at 77.0. Conformational searching and molecular modeling methods are described by Hinkley et al. (*9*).

Collection and Extraction. *P. axillaris* twigs and leaves were collected from the West coast of the South Island, New Zealand, in January 1997 (collection code 970128-27) and from the Nelson region in March 1999 (990327-01). Voucher specimens are lodged in the Plant Extracts Research Unit collection, Crop & Food Research.

Antifungal Assays. Initial biological activity was detected with the *Phytophthora infestans* (Bayer code PHYTIN, late tomato blight) in vitro test (*10*), which was also used to evaluate chromatographic fractions. For this assay, cultures of PHYTIN were maintained on rye seed broth. Assays for growth inhibition were conducted using liquid minimal media. Conidia were harvested by flooding a culture plate with assay medium and scraping with a sterile cell lifter. The conidial suspension was then filtered through two layers of sterile cheesecloth and sterile glass wool to remove hyphal fragments that may be present. The conidial suspensions were then adjusted to 3×10^5 conidia/mL using a hemocytometer. Samples were tested for inhibition of fungal growth in 96-well polystyrene microtiter plates, after dilution to desired concentrations in DMSO so that all wells received 2.5 μL of DMSO. Plates were inoculated by adding 200 μL of the conidial suspension. The incubation time was 72 h. Plates were evaluated for percent inhibition of fungal growth using a nephelometer (Nephelostar Galaxy, BMG Labtechnologies, Offenburg, Germany).

Samples were also evaluated for in vivo control of whole plant fungal infections according to Ricks et al. (*11*). In these assays, extracts or compounds were dissolved in acetone, with serial dilutions then made in acetone to obtain desired concentrations: 6.25, 25, and 100 ppm for pure compounds (**Table 3**). Final treatment volumes were obtained by adding 9 volumes of 0.05% aqueous Tween-20 or 0.01% Triton X-100, depending upon the pathogen. For each pathogen, host plants were grown from seed in a soilless peat-based potting mixture (Metro-Mix) until the seedlings were 10−20-cm tall. These plants were then sprayed to runoff with the test compound. After 24 h, the test plants were inoculated by spraying with an aqueous sporangia suspension of fungus and kept in a dew chamber overnight. The plants were then transferred to the greenhouse until disease developed in the untreated control plants. The pathogens and plants were the following: *Plasmopara viticola* (Bayer code PLASVI, grape downy mildew) on vines (cultivar Carignane); PHYTIN on tomatoes (cultivar Rutgers); *Puccinia graminis tritici* (PUCCRT, wheat stem rust), *Septoria tritici* (SEPTTR, wheat leaf spot), and *Septoria nodorum* (LEPTNO, wheat glume blotch) on wheat (cultivar Yuma); and *Erysiphe graminis tritici* (ERYSGT, wheat powdery mildew) on wheat (cultivar Monon). Disease incidence was measured relative to untreated control plants at 24-h post inoculation and, in some tests, at 96 h (**Table 3**). The *C. albicans* assay has been described previously (*12*).

Bioassay-Directed Isolation of Paxidal (3). Foliage (101 g) was blended with EtOH (1.4 L), then filtered and evaporated to give a green solid (11.3 g). This crude extract exhibited 100% mean disease control of PHYTIN in vivo at 1000 ppm. A sample of the crude extract (4.1 g) was dried onto C18 silica gel (8 g) and loaded onto a C18 column (40 g). Then, 44 fractions were eluted with 21-mL volumes of H_2O/MeCN (1:0, 9:1, 3:1, 1:1, 1:3, 1:9, and 0:1), 1:1 MeCN/$CHCl_3$, and $CHCl_3$. These were combined on the basis of similarity by TLC. Fractions eluted with H_2O/MeCN (9:1 and 3:1) exhibited 100% killing of PHYTIN in vitro at 500 ppm.

One active fraction (0.34 g) eluted with H_2O/MeCN (9:1) was dried onto silica gel (0.7 g) and loaded onto a silica gel column (35 g). Then, 40 column fractions were eluted with 30-mL volumes of cyclohexane/EtOAc (100−25% cyclohexane, in 5% steps). These fractions were combined on the basis of TLC, and fractions eluted with 70%

Table 3. Antifungal Activity of Paxidal (**3**) and Related Compounds **4**–**14** against Crop Diseases in Vivo[a]

compound	dose (ppm)	LEPTNO	PHYTI N	PLASVI	PUCCRT	SEPTTR
3	100	17/18	69/9	51/0	0	7
3	25	0/0	57/18	23/0	0	0
4	100	73	100/3	76/86	77/61	0
4	25	0	82/7	28/0	8/4	0
4	6.25	0	72/0	0/0	0/0	0
5	100	96	100	89	85	0
5	25	73	91	19	15	0
5	6.25	33	74	2	4	0
6	100	60	28	24	0	0
6	25	0	36	4	0	0
6	6.25	0	19	7	0	0
7	100	20	98	70	21	0
7	25	20	67	33	0	0
7	6.25	20	3	9	0	0
8	100	20	99/14	74/57	71/19	0
8	25	0	78/6	61/23	4/0	0
8	6.25	0	47/0	7/0	4/0	0
9	100	0	0	0	7	78
9	25	0	0	0	4	33
9	6.25	0	0	0	0	43
10	100	0	0	0	9	90
10	25	0	0	0	9	17
10	6.25	0	0	0	42	0
11[b]	100	0	0	0	0	11
12[b]	100	0	0	0	0	0
13[b]	100	0	0	0	0	0
14	100	0	58	70	0	0
14	25	0	8	22	0	0
14	6.25	0	-6	4	0	0

[a] Results are % disease inhibition after 24 h, except for some 24 h/96 h results. LEPTNO = *Septoria nodorum* = glume blotch on wheat; PHYTIN = *Phytophthora infestans* = late tomato blight; PLASVI = *Plasmopara viticola* = grape downy mildew; PUCCRT = *Puccinia graminis tritici* = wheat stem rust; SEPTTR = *Septoria tritici* = leaf spot on wheat. None of these compounds showed any activity against *Erysiphe graminis tritici* = powdery mildew on wheat, at 100 ppm or lower. [b] No activity at lower levels.

cyclohexane showed 100% killing of PHYTIN in vitro at 500 ppm. These active fractions were subjected to preparative RPLC (Lichrospher 100 RP-18 column, H_2O/MeCN 1:9 at 3 mL/min) to give paxidal (**3**, 20 mg), characterized below.

Improved Isolation of Paxidal (3) plus 6-Hydroxypaxidals (4) and (5). Dried *P. axillaris* foliage (50 g) was crushed and shaken with $CHCl_3$ (200 mL) at room temperature overnight, then filtered, and the solvent evaporated to give a green gum (4.65 g). Flash vacuum chromatography on silica gel 60H started with CH_2Cl_2, then elution with 5 and 10% EtOAc in CH_2Cl_2 gave crude paxidal (1.5 g), and 20−100% EtOAc in CH_2Cl_2 gave crude mixed hydroxy paxidals (400 mg). To remove the chlorophylls and carotenoids from these fractions, MeCN solutions were passed through short columns of RP C-18, to give the crude products as pale yellow oils. Column chromatography over silica gel on the crude paxidal fraction eluting with 5% EtOAc in CH_2Cl_2 gave paxidal mixed 10:1 with an unidentified dialdehyde as a pale yellow oil (0.96 g). Recrystallization from methanol gave pure paxidal (**3**) as an off-white crystalline solid: mp 120 °C (MeOH); analysis found C 76.33, H 7.05, $C_{24}H_{26}O_4$ requires C 76.19, H 6.88; $[\alpha]^{28}_D$ +11°, $[\alpha]^{28}_{577nm}$ +0.3°, $[\alpha]^{28}_{546nm}$ −10°, $[\alpha]^{28}_{435nm}$ −81°, $[\alpha]^{28}_{405nm}$ −74° (c 0.2, MeOH); UV (MeOH) λ_{max} (ϵ) 280 (18 270) nm; IR (film) ν_{max} 1705, 1680, 1640, 1170 cm^{-1}; ^1H and ^{13}C NMR in **Tables 1** and **2**; EIMS *m*/*z* 378 (M^+, 2), 350 (22), 247 (22), 230 (42), 202 (55), 187 (19), 173 (24), 131 (100), 103 (76), 91 (30), 77 (45).

Silica gel chromatography of the crude hydroxy paxidals with 10−100% EtOAc in CH_2Cl_2 gave initially pure 6β-hydroxypaxidal (**4**, 177 mg) as a pale yellow gum: $[\alpha]^{28}_D$ −2°, $[\alpha]^{28}_{577nm}$ −10°, $[\alpha]^{28}_{546nm}$ −20°, $[\alpha]^{28}_{435nm}$ −90°, $[\alpha]^{28}_{405nm}$ −74° (c 0.2, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 279 (17 360) nm; IR (film) ν_{max} 3464, 2960, 1708, 1686, 1636, 1173 cm^{-1}; ^1H and ^{13}C NMR in **Tables 1** and **2**; EIMS *m*/*z* 394.1769 (M^+, 0.1, calcd for $C_{24}H_{26}O_5$, 394.1780), 376.1681 (M^+-H_2O, 0.4, calcd for $C_{24}H_{24}O_4$, 376.1675), 131 (100).

This was followed by pure 6α-hydroxypaxidal (**5**, 72 mg) as a pale yellow gum: $[\alpha]^{28}_D$ +58°, $[\alpha]^{28}_{577nm}$ +50°, $[\alpha]^{28}_{546nm}$ +47°, $[\alpha]^{28}_{435nm}$ +19°, $[\alpha]^{28}_{405nm}$ −21° (c 0.2, MeOH); UV (MeOH) λ_{max} (ϵ) 278 (13 880) nm; IR (film) ν_{max} 3444, 2960, 2870, 1710, 1686, 1636, 1171 cm^{-1}; ^1H and ^{13}C NMR in **Tables 1** and **2**; EIMS *m*/*z* 376.1682 (M^+-H_2O, 0.3, calcd for $C_{24}H_{24}O_4$, 376.1675), 131 (100).

Synthetic Derivatives. *Diol (**14**)*. $NaBH_4$ (20 mg) was added to a solution of paxidal (**3**) (50 mg) in absolute EtOH (2 mL) and left for 1 h. The solution was concentrated, then 10% HCl (2 mL) was added, and resulting solution was extracted with EtOAc. The organic phase was washed, dried, and concentrated to give the crude diol. Column chromatography over silica gel, eluting with 10−100% EtOAc in CH_2Cl_2 gave the pure diol (**14**, 24 mg, 48%) as a colorless gum: $[\alpha]^{28}_D$ −16°, $[\alpha]^{28}_{577nm}$ −1°, $[\alpha]^{28}_{546nm}$ −3°, $[\alpha]^{28}_{435nm}$ −14°, $[\alpha]^{28}_{405nm}$ −17° (c 0.2, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 280 (34 380) nm; IR (film) ν_{max} 3356, 2928, 1838, 1708, 1637, 1174 cm^{-1}; ^1H and ^{13}C NMR in **Tables 1** and **2**; EIMS *m*/*z* 364.2032 (M^+-H_2O, 1, calcd for $C_{24}H_{28}O_3$ 364.2038), 362.1873 (M^+-H_2O-H_2, 3, calcd for $C_{24}H_{26}O_3$ 362.1882), 131 (100).

*Paxidal Dimethyl Acetal (**8**)*. A solution of paxidal (**3**) (150 mg) in MeOH (5 mL) was run through a column (20 × 20 mm) of DOWEX 50W×8 (100−200 mesh, H^+) ion-exchange resin (prewashed with MeOH) to give the crude dimethyl acetal (172 mg). A sub-sample (50 mg) was chromatographed over silica gel eluted with 5% EtOAc in CH_2Cl_2 to give the pure acetal (**8**, 38 mg, 78%) as a colorless gum: $[\alpha]^{28}_D$ −9°, $[\alpha]^{28}_{577nm}$ −24°, $[\alpha]^{28}_{546nm}$ −38°, $[\alpha]^{28}_{435nm}$ −145°, $[\alpha]^{28}_{405nm}$ −171° (c 0.2, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 280 (13,220) nm; IR (film) ν_{max} 2928, 1711, 1639, 1174 cm^{-1}; ^1H and ^{13}C NMR in **Tables 1** and **2**; EIMS *m*/*z* 392.2001 (M^+-CH_3OH, 3, calcd for $C_{25}H_{28}O_4$, 392.1983), 197 (100).

*1β-Hydroxydial (**6**)*. NaOH (0.5 g) in H_2O (2 mL) was added to a solution of paxidal dimethyl acetal (**8**) (172 mg) in DMSO (4 mL), and the mixture was kept at 55 °C for 20 h. After cooling, the mixture was diluted with H_2O (50 mL) and extracted with EtOAc (3 × 100 mL). The organic phase was washed with H_2O (50 mL), dried, and concentrated to give the crude 1-β-hydroxydial dimethyl acetal (83 mg). This was then dissolved in acetone (4 mL) and H_2O (1 mL), toluenesulfonic acid (10 mg) was added, and the mixture was left at RT for 20 min. Saturated aqueous $NaHCO_3$ (20 mL) was added, and the solution was extracted with EtOAc (3 × 20 mL). The organic phase was washed with H_2O (20 mL), dried, and concentrated to give the crude dial. Column chromatography on silica gel eluting with 5−100% EtOAc in CH_2Cl_2 gave the pure 1β-hydroxydial (**6**, 38 mg, 34% from **8**) as a colorless oil: $[\alpha]^{28}_D$ 25°, $[\alpha]^{28}_{577nm}$ 11°, $[\alpha]^{28}_{546nm}$ 9°, $[\alpha]^{28}_{435nm}$ −26°, $[\alpha]^{28}_{405nm}$ −4° (c 0.2, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 227 (13 980) nm; IR (film) ν_{max} 3460, 2872, 1714, 1682, 1644 cm^{-1}; ^1H and ^{13}C NMR in **Tables 1** and **2**; EIMS *m*/*z* 248.1410 (M^+, 1, calcd for $C_{15}H_{20}O_3$, 248.1412), 91 (100).

*1β-Methoxydial (**7**)*. A solution of 1β-hydroxydial (**6**) (35 mg) in MeI (5 mL) containing silver oxide (10 mg) and 4-Å molecular sieves (10 mg) under N_2 was sonicated for 15 s and then refluxed for 2 h. Filtration and evaporation gave a mixture of an acetal and the required methyl ether, which was separated by column chromatography on silica gel eluting with 0−20% EtOAc in CH_2Cl_2 to give 1β-methoxydial (**7**, 5 mg, 14%) as a colorless oil: $[\alpha]^{28}_D$ 64°, $[\alpha]^{28}_{577nm}$ 57°, $[\alpha]^{28}_{546nm}$ 56°, $[\alpha]^{28}_{435nm}$ 53°, $[\alpha]^{28}_{405nm}$ 98° (c 0.2, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 225 (14 400), 280 (3900) nm; IR (film) ν_{max} 2934, 1715, 1682, 1096 cm^{-1}; ^1H and ^{13}C NMR in **Tables 1** and **2**; EIMS *m*/*z* 262.1576 (M^+, 5, calcd for $C_{16}H_{22}O_3$ 262.1569), 91 (100). This was followed by recovered compound **6** (15 mg).

*6α-Hydroxypaxidal Dimethyl Acetal (**9**)*. A solution of 6α-hydroxypaxidal (**5**) (48 mg) in MeOH (5 mL) was run through a column (20 × 20 mm) of DOWEX 50W×8 (100−200 mesh, H^+) ion-exchange resin (pre-washed with MeOH) to give the crude dimethyl acetal.

Purification by column chromatography over silica gel eluting with increasing proportions of EtOAc in CH_2Cl_2 gave the pure 6α-hydroxypaxidal dimethyl acetal (**9**, 41 mg, 80%) as a colorless oil: $[\alpha]^{28}_{D}$ 31°, $[\alpha]^{28}_{577nm}$ 22°, $[\alpha]^{28}_{546nm}$ 14°, $[\alpha]^{28}_{435nm}$ −57°, $[\alpha]^{28}_{405nm}$ −69° (c 0.2, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 280 (18 040) nm; IR (film) ν_{max} 3450, 2923, 1705, 1640, 1177 cm^{-1}; ^1H and ^{13}C NMR in **Tables 1** and **2**; EIMS *m/z* 408.1939 (M^+-CH_3OH, 3, calcd for $C_{25}H_{28}O_5$ 408.1937), 131 (100).

*Acetal (**11**)*. To a solution of the acetal (**9**) (32 mg) in DMSO (0.6 mL), aqueous NaOH (25%, 0.3 mL) was added, and the mixture was left at 55 °C for 20 h. After cooling, the reaction mixture was diluted with H_2O (30 mL) and extracted with EtOAc (3 × 50 mL). The organic phase was washed with H_2O (30 mL), dried, and concentrated to give the acetal (**11**, 19 mg, 60%) as a colorless oil: $[\alpha]^{28}_{D}$ 112°, $[\alpha]^{28}_{577nm}$ 107°, $[\alpha]^{28}_{546nm}$ 110°, $[\alpha]^{28}_{435nm}$ 107°, $[\alpha]^{28}_{405nm}$ 133° (c 0.2, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 270 (2010) nm; IR (film) ν_{max} 3460, 2896, 971 cm^{-1}; ^1H and ^{13}C NMR in **Tables 1** and **2**; EIMS *m/z* 279.1603 (M^+-OCH_3, 33, calcd for $C_{16}H_{23}O_4$, 279.1596), 200 (100).

*Acetal (**13**)*. A solution of acetal (**11**) (19 mg) in MeI (5 mL) containing silver oxide (10 mg) and 4-Å molecular sieves (10 mg) under N_2 was sonicated for 15 s and then refluxed for 2 h. Filtration and evaporation gave the monomethyl ether, which was purified by column chromatography on silica gel eluting with 0−20% EtOAc in CH_2Cl_2 to give acetal (**13,** 15 mg, 70%) as a colorless oil: $[\alpha]^{28}_{D}$ 187°, $[\alpha]^{28}_{577nm}$ 186°, $[\alpha]^{28}_{546nm}$ 199°, $[\alpha]^{28}_{435nm}$ 265°, $[\alpha]^{28}_{405nm}$ 327° (c 0.2, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 280 (972) nm; IR (film) ν_{max} 3370, 1640 cm^{-1}; ^1H and ^{13}C NMR in **Tables 1** and **2**; EIMS *m/z* 293.1753 (M^+-OCH_3, 32, calcd for $C_{17}H_{25}O_4$ 293.1757), 45 (100).

*6-Ketopaxidal (**12**)*. PCC (3 × 10 mg) was added to a solution of the 6α-hydroxypaxidal acetal (28 mg) in dry CH_2Cl_2 (3 mL) over 30 min. The solution was put down a short column of silica, and 10% EtOAc in CH_2Cl_2 eluted the pure ketone (**12**, 17 mg, 61%) as a colorless oil: $[\alpha]^{28}_{D}$ 97°, $[\alpha]^{28}_{577nm}$ 89°, $[\alpha]^{28}_{546nm}$ 86°, $[\alpha]^{28}_{435nm}$ 17°, $[\alpha]^{28}_{405nm}$ −92° (c 0.2, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 280 (25 186) nm; IR (film) ν_{max} 3370, 1705, 1673, 1640 cm^{-1}; ^1H and ^{13}C NMR in **Tables 1** and **2**; EIMS *m/z* 438.2034 (M^+, 1, calcd for $C_{26}H_{30}O_6$ 438.2034), 131 (100).

*Dihydrocinnamate (**10**)*. A solution of the acetal (**9**) (17 mg) in MeOH (4 mL) was stirred with Pd on C (5%, 5 mg) under an atmosphere of H_2 for 2 h. The suspension was filtered through Celite and evaporated to give the pure dihydrocinnamate (**10**, 16 mg, 97%) as a colorless oil: $[\alpha]^{28}_{D}$ 82°, $[\alpha]^{28}_{577nm}$ 75°, $[\alpha]^{28}_{546nm}$ 73°, $[\alpha]^{28}_{435nm}$ 50°, $[\alpha]^{28}_{405nm}$ 72° (c 0.2, $CHCl_3$); UV (MeOH) end adsorption; ^1H and ^{13}C NMR in **Tables 1** and **2**; EIMS *m/z* 440.2203 (M^+-H_2, 1, calcd for $C_{26}H_{32}O_6$ 440.2199), 91 (100).

RESULTS AND DISCUSSION

New Natural Products. An ethanol extract of *P. axillaris* foliage showed in vitro activity against *Phytophthora infestans* (late blight of tomatoes and potatoes, PHYTIN) in high throughput screening. This activity was confirmed as interesting when the extract exhibited 100% control of PHYTIN in vivo at 1000 ppm. The in vitro PHYTIN assay was used to direct the isolation of the fungicidal compounds. Reversed-phase (RP) C_{18} column chromatography followed by silica gel column chromatography gave an active fraction, which was subjected to preparative RPLC to give the main active compound **3**. This compound was of medium-to-low polarity, and it was found that a chloroform extract of foliage gave a better yield of **3** (1.9 wt % of dry foliage). This also gave two related compounds, **4** (0.35%) and **5** (0.14%), showing even greater activity against PHYTIN than **3** (see below).

The structure of **3** was proven by a combination of NMR experiments. The ^{13}C NMR spectrum (**Table 1**) showed 22 carbon signals, whereas the mass spectrum and elemental composition suggested the molecular formula $C_{24}H_{26}O_4$. This deficit was accounted for by the presence of two pairs of equivalent carbons in a trans cinnamate group, shown by the aromatic signals of a monosubstituted benzene ring, characteristic double bond ^1H signals at 6.42 and 7.69 (both d, J 16 Hz), and a ^{13}C signal at 166.1 for the ester carbonyl (**Tables 1** and **2**). Other NMR signals (**Tables 1** and **2**) were very similar to those reported for 9-deoxymuzigadial (**2**) (*13*), especially the dialdehyde and exocyclic methylene signals. 2-D NMR studies (Supporting Information), especially an HMBC correlation between H1 and the ester carbonyl, showed that the cinnamate was linked to the sesquiterpene at C1. The relative stereochemistry was proven by NOE interactions between axial protons H1, H3, H5, and H9 (**Figure 1**). The absolute stereochemistry was assumed to be the same as that for polygodial (**1**) and 9-deoxymuzigadial (**2**) in *P. colorata* (*7, 14*), giving the proposed structure **3**, a new compound for which we propose the name paxidal.

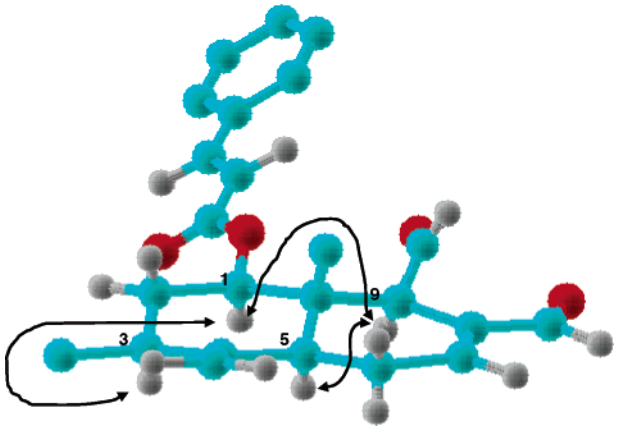

Figure 1. Predicted most stable conformation of paxidal (**3**) from molecular modeling, showing key NOE interactions (Me14, Me15, and phenyl protons omitted for clarity)

The two other compounds isolated from *P. axillaris* had NMR spectra very similar (**Tables 1** and **2**) to that of paxidal (**3**), and MS showed them both to have one more oxygen than **3**. Both new compounds showed oxygenated methine signals (4.81/4.76 ppm) coupled to H5 and H7 (**Table 2** and Supporting Information), indicating a 6-CHOH group. The minor compound showed a diaxial coupling of 9.5 Hz between H6 and H5 which, together with NOE interactions between the H6 proton and Me15, showed this compound to be the 6α-hydroxypaxidal (**5**). The other compound showed only a weak H6−H5 coupling, and an NOE interaction between the H6 and one H13, so this is 6*β*-hydroxypaxidal (**4**).

Compounds **3**−**5** are new examples of the rare muzigadial skeleton and the first with oxygenation at C1 or C6 (*8*). The only examples of drimane dialdehydes oxygenated at C1 are 1*β*-*p*-coumaroyloxypolygodial (**15**) and a few simple derivatives, isolated from three different *Drimys* species (also in the family Winteraceae) (*15*−*18*). The NMR signals for H1 and C1 of **15** (*18*) were similar to those for paxidal (**3**, **Tables 1** and **2**). Drimane dialdehydes oxygenated at C6 are more common, but none oxygenated at both C1 and C6 have been reported previously (*8*).

We have found that dialdehydes **1** and **2** are quite unstable on storage, as have others (*19*), but compounds **3**−**5** were stable under the same conditions. This may be due to steric congestion around the C11 aldehyde, shown by molecular modeling (**Figure 1**), which restricts access to this reactive center. There is a change in the average conformation about the C9 to C11 bond, since the H9 to H11 couplings in compounds **1** and **2** are about 4.5 Hz (*7, 13*), whereas the corresponding couplings in **3**−**5** are around 2 Hz (**Table 2**). The stability of these compounds

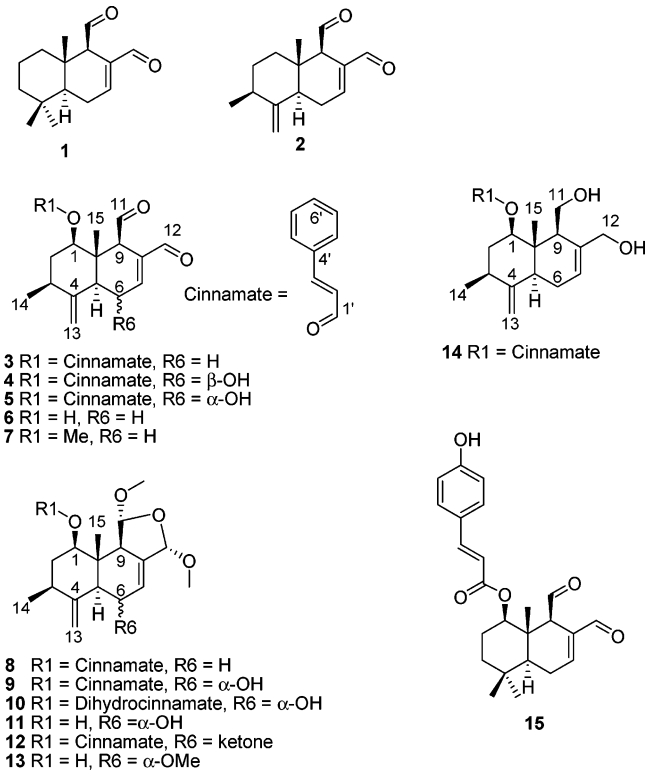

Figure 2.

3−**5** and their different fungicidal activities (see below) encouraged us to synthesize derivatives of the natural products for testing.

Synthesis of Derivatives. The first set of derivatives was based on the most abundant paxidal (**3**), probing the importance of the obvious functional groups, that is, the dialdehyde and cinnamate moieties. Reduction of the dialdehyde **3** to the diol **14** was readily achieved with sodium borohydride. Compound **14** showed NMR signals (**Tables 1** and **2**) around the diol moiety similar to the corresponding signals in the naturally occurring diol derivative of polygodial (*20*).

The dialdehyde functionality of paxidal (**3**) was converted to a dimethyl acetal using ion-exchange resin. Only one major diastereoisomer **8** was formed, although four are possible. The same treatment of polygodial (**1**) has been found to give two dimethyl acetals, with the major product having both methoxy groups α, and the minor product having 11α and 12*β* (*19*). The paxidal dimethyl acetal **8**, and the other dimethyl acetals **9**−**13**, all showed NOE interactions between H11 and Me15, suggesting 11α methoxy groups. No NOE interaction was observed between H11 and H12, but molecular modeling suggested that these protons would be separated by over 3.5 Å for either geometry at C12. We propose 12α methoxy groups in dimethyl acetals **8**−**13**, on the basis of the similarity of our ^{1}H NMR data (**Table 2**) to that reported for the corresponding polygodial 11α,12α-dimethyl acetal (*19*). We found that even the small amount of acid present in some samples of deuteriochloroform was enough to cause hydrolysis of the acetal **8** back to the free dialdehyde.

Protection of the dialdehyde in dimethyl acetal **8** allowed removal of the highly hindered cinnamate ester by hydrolysis under the relatively drastic conditions of heating a dimethylsulfoxide solution with 25% potassium hydroxide to 55 °C overnight. This gave the 1*β*-hydroxy acetal, which was deprotected by mild acid hydrolysis to give the 1*β*-hydroxydial (**6**). The NMR spectra of **6** (**Tables 1** and **2**) showed the expected features, especially H1 appearing at higher field than in the cinnamate esters. Methylation of the highly hindered alcohol (**6**) was achieved, albeit in a low yield, to give the methoxy ether (**7**). Unfortunately all attempts to reduce the conjugated 7−8 double bond of paxidal (**3**) proved unsuccessful. Catalytic hydrogenation gave complex mixtures with the aldehyde groups and the side-chain double bond reduced, but with the 7−8 double bond intact. Dissolving metal reductions, and other 1,4-reducing agents, also gave complex mixtures.

The next series of derivatives were formed from the less abundant but more active (see below) 6α-hydroxypaxidal (**5**). First the dimethyl acetal **9** was formed using the same ion-exchange resin conditions as before. Hydrolysis of the cinnamate group by the same method as before gave the diol **11**. Methylation led to a single monomethyl product, which was shown to be the 6-methoxy product (**13**) by an HMBC correlation between C6 and one methoxy signal, plus the downfield shift of the C6 signal (**Table 1**). This regioselectivity was explained by the much less hindered environment of C6. Other attempts to differentiate between C1 and C6 led to the formation of the 6-keto product (**12**), but this proved unstable to the strongly basic conditions needed to hydrolyze the cinnamate ester to take the route further. Variation in the cinnamate group was achieved by catalytic hydrogenation of dimethyl acetal **9** to give the dihydrocinnamate (**10**), with no sign of reduction of the 7−8 double bond.

Fungicidal Activities. The natural products **3**−**5** and synthetic derivatives **6**−**14** were tested in vivo against some important food crop pathogens (**Table 3**). In addition to the activity of paxidal (**3**) against PHYTIN (late blight of tomatoes and potatoes), which was used to direct its isolation, compound **3** was also active against *Plasmopara viticola* (PLASVI, grape downy mildew). However, inhibition of both of these diseases fell off after 4 days (**Table 3**).

This initial result prompted us to prepare and test synthetic derivatives of paxidal (**3**), plus the co-occurring natural products**.** The natural 6-hydroxy compounds **4** and **5** were more active against PHYTIN and showed a broader spectrum of activity, inhibiting *Septoria nodorum* (LEPTNO, glume blotch on wheat) and *Puccinia graminis tritici* (PUCCRT, wheat stem rust, **Table 3**). The synthetic paxidal derivatives **6** and **14** also showed interesting antifungal activities (**Table 3**), so we synthesized derivatives of 6α-hydroxypaxidal (**5**), the most active natural product.

Surprisingly, even though paxidal dimethyl acetal (**8**) had shown a potency and spectrum of activity similar to that of 6α-hydroxypaxidal (**5**), 6α-hydroxypaxidal dimethyl acetal (**9**) showed a different spectrum of activity (**Table 3**). Compound **9**, and its dihydrocinnamate analogue **10**, were inactive against PHYTIN, LEPTNO, and PUCCRT, but were active against *Septoria tritici* (SEPTTR, leaf spot on wheat). This change in selectivity may be related to the greater stability of 6α-hydroxypaxidal dimethyl acetal (**9**) to mild acid hydrolysis, compared to paxidal dimethyl acetal (**8**). This was shown by the greater stability of 6α-hydroxypaxidal dimethyl acetal (**9**) in deuteriochloroform, compared to paxidal dimethyl acetal (**8**).

Considering the potencies of the antifungal activities overall (**Table 3**), some structure−activity relationships can be suggested. Substitution and orientation at C6 is important, with antifungal potencies in the following order: 6α-hydroxy **5** > 6*β*-hydroxy **4** > 6-deoxy **3**; and 6α-hydroxy **9** > 6-keto **12**. A bulky and/or low polarity substituent at C1 is also important: methoxy **7** > cinnamate **3** > hydroxy **6**; and cinnamate **9** ≈ dihydrocinnamate **10** > hydroxy **11**. The free dialdehyde is not

essential for antifungal activity: dimethyl acetal **8** > dialdehyde **3** > diol **14**.

This last finding was surprising, since the general biological activity of polygodial (**1**) is ascribed to the unsaturated dialdehyde functionality and its reactivity toward biological nucleophiles (*8*). Given the sensitivity of the acetal **8** to acid-catalyzed hydrolysis (see above), the activity of **8** may be due to a sort of controlled release of **3**, that is, **8** is a pro-drug, itself not very active but slowly releasing the potent **3**.

Sesquiterpene dialdehydes such as **1** are especially active against the yeasts *C. albicans* and *Saccharomyces cerevisiae*. However, cinnamate-substituted sesquiterpene dialdehydes **3**−**5** showed no activity against *C. albicans* in a disk diffusion assay in which compounds **1** and **2** showed strong activity (all at 60 μg/ disk). On the other hand, compounds **1** and **2** are not active against PHYTIN, since an extract of *P.* colorata rich in these compounds (*6*) was not active against this plant pathogen. There do not seem to be any other reports of sesquiterpene dialdehydes being active against the important food crop pathogens studied here (*8*). Therefore the sesquiterpene dialdehyde cinnamate paxidal (**3**) and its derivatives might be the basis of a new class of crop protection agents, if stability problems could be overcome.

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Supporting Information Available: Tables of 2D NMR correlation data for compounds **3**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Irvine, N. M.; Ricks, M. J.; Ross, R.; Bryan, K.; Klittich, C. J. R. Preparation of fungicidal 4-(2-aminopyridin-4-yl)-*N*-phenyl-1,3,5-triazin-2-amine deriviatives. In *CAN 142: 392441*; Dow Agrosciences LLC: PCT Int. Appl., 2005033095, 2005.

(2) Yamaguchi, I.; Fujimura, M. Recent topics on action mechanisms of fungicides. *J. Pestic. Sci.* **2005**, *30*, 67−74.

(3) Perry, N. B. Natural products from New Zealand plants. In *Agrochemical Discovery: Insect, Weed, and Fungal Control*; Baker, D. R., Umetsu, N. K., Eds.; American Chemical Society: Washington, DC 2001; pp 48−61.

(4) Allan, H. H. *Flora of New Zealand. Indigenous Tracheophyta. Psilopsida, Lycopsida, Filicopsida,* Gymnospermae, Dicotyledones; DSIR: Wellington, 1961; Vol. I.

(5) Salmon, J. T. *The native trees of New Zealand*, revised ed.; Reed Methuen: Auckland, 1986.

(6) Perry, N. B.; Foster, L. M.; Lorimer, S. D. Infraspecific variation of insecticidal sesquiterpene dialdehydes in *Pseudowintera colorata*. *Phytochemistry* **1996**, *43*, 1201−1203.

(7) McCallion, R. F.; Cole, A. L. J.; Walker, J. R. L.; Blunt, J. W.; Munro, M. H. G. Antibiotic Substances from New Zealand plants. II. Polygodial, an anti-candida agent from *Pseudowintera colorata*. *Planta Med.* **1982**, *44*, 134−138.

(8) Jansen, B. J. M.; de Groot, A. Occurrence, biological activity and synthesis of drimane sesquiterpenoids. *Nat. Prod. Rep.* **2004**, *21*, 449−477.

(9) Hinkley, S. F.; Perry, N. B.; Weavers, R. T. Confirmation of structure and absolute stereochemistry of 9-epi-beta-caryophyllene from *Dacrydium cupressinum*. *Phytochemistry* **1994**, *35*, 1489−1494.

(10) Adou, E.; Williams, R. B.; Schilling, J. K.; Malone, S.; Meyer, J.; Wisse, J. H.; Frederik, D.; Koese, D.; Werkhoven, M. C. M.; Snipes, C. E. Cytotoxic diterpenoids from two lianas from the Suriname rainforest. *Bioorg. Med. Chem.* **2005**, *13*, 6009−6014.

(11) Ricks, M. J.; Dent, W. H.; Rogers, R. B.; Yao, C.; Nader, B. S.; Miesel, J. L.; Fitzpatrick, G. M.; Meyer, K. G.; Niyaz, N. M.; Morrison, I. M.; Gajewski, R. P. Preparation, compositions and use of heterocyclic aromatic amino acids as fungicides. In *CAN 134: 207831*; Dow Agrosciences LLC: PCT Int. Appl., 2001014339, 2001.

(12) Lorimer, S. D.; Barns, G.; Evans, A. C.; Foster, L. M.; May, B. C. H.; Perry, N. B.; Tangney, R. S. Cytotoxicity and antimicrobial activity of plants from New Zealand's subantarctic islands. *Phytomedicine* **1996**, *2*, 327−333.

(13) Al-Said, M. S.; El-Khawaja, S. M.; El-Feraly, F. S.; Hufford, C. D. 9-deoxy drimane sesquiterpenes from *Canella winterana*. *Phytochemistry* **1990**, *29*, 975−977.

(14) Gerard, P. J.; Perry, N. B.; Ruf, L. D.; Foster, L. M. Antifeedant and insecticidal activity of compounds from *Pseudowintera colorata* (Winteraceae) on the webbing clothes moth, *Tineola bisselliella* (Lepidoptera: Tineidae) and the Australian carpet beetle, *Anthrenocerus australis* (Coleoptera: Dermestidae). *Bull. Entomol. Res.* **1993**, *83*, 547−552.

(15) Cechinel Filho, V.; Schlemper, V.; Santos, A. R. S.; Pinheiro, T. R.; Yunes, R. A.; Mendes, G. L.; Calixto, J. B.; Delle Monache, F. Isolation and identification of active compounds from *Drimys winteri* barks. *J. Ethnopharmacol.* **1998**, *62*, 223−227.

(16) Malheiros, A.; Filho, V. C.; Schmitt, C. B.; Santos, A. R. S.; Scheidt, C.; Calixto, J. B.; Monache, F. D.; Yunes, R. A. A sesquiterpene drimane with antinociceptive activity from *Drimys winteri* bark. *Phytochemistry* **2001**, *57*, 103−107.

(17) Ferreto, L.; Ciccio, J. F.; Castro, V.; Andrade, R. Drimane derivatives from *Drimys granadensis* L. f. (Winteraceae). *Spectroscopy* **1988**, *6*, 133−136.

(18) Vichnewski, W.; Kulanthaivel, P.; Herz, W. Drimane derivatives from *Drimys brasiliensis*. *Phytochemistry* **1986**, *25*, 1476−1478.

(19) Tozyo, T.; Yasuda, F.; Nakai, H.; Tada, H. Cytotoxic hydroxypolygodials. X-ray molecular structure of (1R, 3S, 5aS,-9aS, 9bR)-1, 3, 5, 5a, 6, 7, 8, 9, 9b-decahydro-1,3-dimethox y-6, 6, 9a-trimethylnaphtho[1,2-c]furan. *J. Chem. Soc., Perkin Trans. 1* **1992**, 1859−1866.

(20) Brown, G. D. Drimendiol, a sesquiterpene from *Drymis winteri*. *Phytochemistry* **1994**, *35*, 975−977.

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