5750 J. Agric. Food Chem. 2009, 57, 5750–5755
DOI:10.1021/jf900674y

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Antifungal Activity of CHE-23C, a Dimeric Sesquiterpene from *Chloranthus henryi*

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An antifungal compound was isolated from methanol extracts of stems and roots of *Chloranthus henryi* Hemsl. using ethyl acetate extraction and various chromatographic techniques. On the basis of spectroscopic analyses including mass and various NMR, the structure of the compound was identified as a dimeric sesquiterpene, CHE-23C. The compound showed potent antifungal activities (MICs = 1−32 μg/mL) in vitro against various phytopathogenic fungi such as *Alternaria kikuchiana*, *Botrytis cinerea*, *Colletotrichum lagenarium*, *Magnaporthe grisea*, *Pythium ultimum*, and *Phytophthora infestans*. In particular, it exhibited 91 and 100% disease-control activity in vivo against tomato late blight (*P. infestans*) and wheat leaf rust (*Puccinia recondita*) at concentrations of 33 and 100 μg/mL, respectively. The disease-control activity of this compound was stronger than that of the commercially available fungicide chlorothalonil, but weaker than that of dimethomorph. Therefore, the compound might serve as an interesting lead to develop effective antifungal agents.

KEYWORDS: *Chloranthus henryi*; CHE-23C; antifungal activity; *Phytophthora infestans*; *Puccinia recondita*

INTRODUCTION

Considerable crop losses are caused by fungal diseases each year, and many agrochemicals have long been used to minimize these losses in crop production (*1*). In contrast, the remaining toxicity and environmental pollution, which are caused by the repeated use of agrochemicals, and the emergence of resistance to commercially available fungicides have become significant problems (*2*−*4*). Although many fungicides for various targets from natural products and chemical synthesis have been reported (*5*−*15*) and some of them are available on the market (*11*−*15*), the development of fungicides having low toxicity, high selectivity, durability, and activity against strains resistant to fungicides used at present is strongly desired.

Chloranthus henryi (Chloranthaceae) is a perennial herb that is distributed widely in the central region, east coast, and southern region of China (*16*) and restrictedly in the eastern part of Taiwan (*17*). This plant has long been recognized to be useful for removing toxic substances from the body in Chinese folk medicine (*18*). Recently, novel sesquiterpenoids (*19*, *20*) and diterpenoids (*21*) isolated from *C. henryi* were reported to represent antitumor activities against HeLa and K562 human tumor cell lines. In addition, novel eudesmane-type sesquiterpenes and germacrane-type sesquiterpenes isolated from the leaves and stems of *C. henryi* have been shown to be blockers of tyrosinase, which can be clinically useful for the treatment of some dermatological disorders associated with melanin hyperpigmentation (*22*). Although several sesquiterpenoids (*19*−*23*) and ditepenoids (*21*) have been isolated from *C. henryi*, sesquiterpenoids with antifungal activity against various plant pathogens have not been reported from this species.

In the course of searching for bioactive compounds against fungal diseases in plants from plant extracts, a sesquiterpene compound was found in the methanol extract of the stems and roots of *C. henryi* Hemsl. Here, we describe the isolation and structure elucidation of a dimeric sesquiterpene CHE-23C and its antifungal activities against various phytopathogenic fungi in vitro and in vivo.

MATERIALS AND METHODS

General Experimental Procedures. High-performance liquid chromatography (HPLC) was conducted using a Watchers 120 ODS-AP column (4.6 × 250 mm, 5 μm, Daiso Co., Tokyo, Japan) in an Agilent model 1100 HPLC equipped with a Agilent model G1312A binary pump and G1328B photodiode array detector (Agilent Technologies, Santa Clara, CA). Ultraviolet (UV) and electrospray ionization (ESI) mass spectral data were recorded on Hewlett-Packard model 8453 and 5989A (Agilent Technologies) spectrophotometers, respectively. Melting point was determined with a Fisher-Johns melting point apparatus (Electrothermal Engineering Ltd.). Optical rotation was measured using a DIP-370 Polarimeter (JASCO Co., Tokyo, Japan). Column chromatographies were performed in silica gel 60 (230−400 mesh, Merck, Darmstadt, Germany) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden).

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 Published on Web 06/15/2009

Thin-layer chromatography (TLC) was carried out on precoated silica gel glass plate (60 F_{254}, Merck). All other chemicals were of the highest grade available and used without further purification.

NMR spectra including ^{1}H and ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) were recorded on a Varian Unity 300 NMR spectrometer (Varian, Palo Alto, CA) using DMSO-d_6. In addition, two-dimensional NMR spectra including ^{1}H−^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC) were measured on a Varian Unity 500 NMR spectrometer.

Plant Material. The dried stems and roots of *C. henryi* were purchased from Korea Plant Extract Bank, which have collections of diverse plant resources obtained from domestic and foreign sources, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, Korea. A voucher specimen has been deposited under no. KRIBB-CHE-23C at KRIBB.

Microorganisms and Culture Media. All of the phytopathogenic fungi used are field isolates provided from Korea Research Institute of Chemical Technology (Daejeon, Korea) and Rural Development Administration (Suwon, Korea) and included *Alternaria kikuchiana*, *Botrytis cinerea*, *Colletotrichum lagenarium*, *Fusarium oxysporum*, *Magnaporthe grisea*, *Phytophthora infestans*, *Pythium ultimum*, and *Rhizoctonia solani*. Among them, *P. infestans* was cultured on V-8 juice agar (200 mL of V-8 juice, 3 g of $CaCO_3$, 15 g of agar, and 1 L of DW) at 20 °C, and the other fungi were incubated on potato dextrose agar (Difco) at 25 °C. In addition, human pathogenic fungi *Candida* spp. except for *C. albicans* A207 and *Cryptococcus neoformans* were from the American Type Culture Collection (ATCC) and cultured on Sabouraud broth (Difco) at 35 °C for 24−48 h.

Extraction and Isolation of a Bioactive Compound. The dried stems and roots of *C. henryi* (3.9 kg) were ground and extracted three times with methanol (15 L) at room temperature for 7 days. The methanol extract was filtered and evaporated in vacuo, and then the crude extracts (329.5 g) were suspended in water (1 L). The suspension was extracted three times with ethyl acetate (0.5 L). The organic layer (73.8 g) was subjected to silica gel column chromatography (Kieselgel 60, 230−400 mesh, Merck, 100 × 600 mm) and eluted stepwise with a gradient of *n*-hexane/$CHCl_3$ (8:2, 6:4, 4:6, 2:8, 0:10, v/v, 1 L of each) and $CHCl_3$/EtOAc (8:2, 6:4, 4:6, 2:8, v/v, 1 L of each). Each eluent was concentrated in vacuo, dissolved in dimethyl sulfoxide (DMSO), and tested against *P. ultimum* using an agar diffusion method. The active fractions (*n*-hexane/$CHCl_3$, 6:4 to $CHCl_3$/EtOAc, 6:4, v/v) were collected, evaporated (34.2 g), and chromatographed on silica gel column (Kieselgel 60, 230−400 mesh, Merck, 55 × 350 mm) by eluting with *n*-hexane/EtOAc (5:5, v/v, 2 L). The resulting fractions (fractions 6−8 among 10 fractions, 8.4 g) were further purified by Sephadex LH-20 column (Lipophilic LH-20, Amersham Biosciences, 17 × 1200 mm) eluting with methanol. The eluents containing antifungal activity against *P. ultimum* were collected and evaporated in vacuo and then crystallized (*n*-hexane/EtOAc, 1:4) to give CHE-23C (650.4 mg) as a white powder. The purity of the compound was determined by HPLC with a C_{18} column (4.6 × 250 mm, 5 μm, Watchers 120 ODS-AP). The column was eluted with a mixture of CH_3OH/H_2O (80:20, v/v) at a flow rate of 1 mL/min. Through the HPLC, a single peak with a retention time of 6.2 min was detected by a UV detector at 254 nm. In addition, to increase the yield of the purification process, the purification steps of antifungal compound were improved by conducting the extraction with chloroform/methanol (4:1) at room temperature for 18 h, silica gel column chromatography with hexane/EtOAc (1:1), C_{18} silica column chromatography, and crystallization (*n*-hexane/EtOAc, 1:4). The purity of the isolated compound was confirmed by HPLC.

Determination of in Vitro Antifungal Activity. During the purification steps, antifungal activities of the fractions obtained from each step were determined by agar diffusion method (*24*, *25*). The potato dextrose agar plates for bioassay were prepared as two separate layers as follows. The mycelial suspensions of test fungi were added into overlay medium containing 0.8% potato dextrose soft agar. The base medium with a solidified potato dextrose agar was then overlaid with the overlay medium containing test fungi. After solidification of the overlay medium, the plates were used in bioassay. Sterile stainless steel cylinders (8 mm outer diameter and 10 mm length, Fisher Co.) were placed on the surface of potato dextrose agar plates seeded with mycelial suspension of various fungi to be tested, and equal aliquots of the methanol extract obtained from *C. henryi* were loaded onto the sterile cylinder and incubated at 25 °C for 48−72 h. The diameter of the inhibitory zone shown on plates was measured and expressed in millimeters. The fractions to be tested were evaporated and dissolved in DMSO, and 50 μL of a 5 mg/mL solution was used to examine the antifungal activity. In bioassay, DMSO was used as a negative control to assess any effect of antifungal activity by DMSO itself. The antifungal activities of active fractions obtained from further purification

Table 1. Yield of CHE-23C Obtained by the Improved Purification Steps

method	initial amount of *C. henryi* (g)	amount of purified CHE-23C (mg)	yield (%)
previous method	3900	650.4	0.017
improved method[a]	180	192	0.106

[a] Improved method indicates the method described under Materials and Methods.

Table 2. ^{1}H and ^{13}C NMR Spectral Data of CHE-23C in DMSO-d_6

position	δ_H	δ_C (DEPT)	HMBC
1	1.88 (m)	25.4 (CH)	C-4, C-5, C-9, C-10
2α	0.89 (m)	15.4 (CH_2)	C-1, C-3, C-4, C-10
2β	0.21 (q, 3.9, 3.0, 3.6)		C-1, C-3, C-4, C-5, C-10
3	1.90 (m)	24.1 (CH)	C-4, C-5
4		140.4 (C)	
5		132.4 (C)	
6	3.89 (d, 3.0)	40.2 (CH)	C-4, C-5, C-7, C-8, C-8′
7		144 (C)	
8		200.3 (C)	
9	3.72 (d, 3.0)	79 (CH)	C-1, C-8, C-10, C-14
10		50.9 (C)	
11		133.3 (C)	
12		170.3 (C)	
13	1.72 (s)	19.4 (CH_3)	C-6, C-7, C-8, C-11, C-12, C-8′
14	0.93 (s)	15.6 (CH_3)	C-1, C-4, C-5, C-9, C-10
15α	2.76 (br d, 15.9)	24.9 (CH_2)	C-3, C-4, C-5, C-8′, C-9′, C-10′
15β	2.40 (tt, 5.4, 4.5, 4.2, 5.7)		C-4, C-5, C-8′, C-9′, C-10′
1’	1.55 (ddd, 4.2, 3.6, 4.2)	24.8 (CH)	C-2′, C-3′, C-4′, C-9′, C-10′
2′α	0.65 (m)	11.9 (CH_2)	C-1′, C-3′, C-4′, C-10′
2′β	1.13 (q, 3.9, 4.2, 6.0)		C-1′, C-3′, C-4′, C-10′
3′	1.37 (ddd, 3.6, 2.7, 3.0)	27.9 (CH)	C-1′, C-4′, C-5′, C-10′, C-15′
4’		75.7 (C)	
5′	1.61 (dd, 6.0, 6.0)	60.4 (CH)	C-4′, C-6′, C-9′, C-10′, C-14′
6′α	2.29 (dd, 5.4, 6.0)	22.5 (CH_2)	C-5′, C-7′, C-10′, C-11′
6′β	2.72 (dd, 4.8, 4.5)		C-4′, C-5′, C-7′, C-8′, C-11′
7′		172.5 (C)	
8′		92.7 (C)	
9′	1.68 (d, 4.8)	55.5 (CH)	C-4, C-6, C-15, C-5′, C-8′, C-10′, C-14′
10′		44.2 (C)	
11′		122.7 (C)	
12′		170.9 (C)	
13′α	4.61 (d, 12.9)	54.4 (CH_2)	C-7′, C-11′, C-12′, C-f
13′β	4.82 (d, 12.6)		C-7′, C-11′, C-12′, C-f
14’	0.79 (s)	26.2 (CH_3)	C-15, C-1′, C-2′, C-5′, C-9′, C-10′
15′α	3.67 (d, 10.8)	70.5 (CH_2)	C-3′, C-4′, C-5′, C-a
15′β	4.10 (d, 11.7)		C-3′, C-4′, C-5′, C-a
a		167.0 (C)	
b		128.1 (C)	
c	6.81 (dd, 2.1, 1.8)	137.1 (CH)	C-a, C-d, C-e
d	1.79 (d, 4.2)	14.2 (CH_3)	C-a, C-b, C-c
e	1.80 (s)	12.0 (CH_3)	C-a, C-b, C-c
f		169.9 (C)	
g	2.03 (s)	20.2 (CH_3)	C-13′, C-f
12-OMe	3.60 (s)	51.9 (C)	C-12
9-OH	5.77 (d, 4.5)		
4′-OH	4.49 (s)		C-3′, C-4′, C-5′

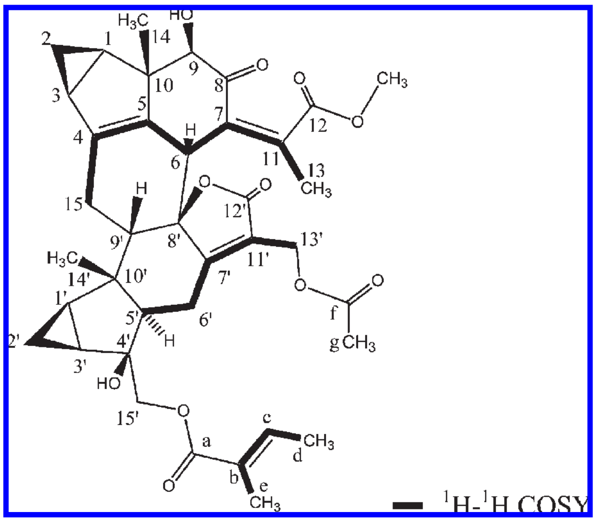

Figure 1. Structure of CHE-23C isolated from *Chloranthus henryi*.

steps were determined by using the same method as described above except for *P. ultimum* as a test organism.

The minimum inhibitory concentrations (MICs) of CHE-23C against various phytopathogenic and human pathogenic fungi were determined by agar dilution method (*26*) using potato dextrose agar for filamentous fungi and a slight modification of the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) method (*27*) for yeast using Sabouraud dextrose broth. CHE-23C dissolved in DMSO was added into the molten potato dextrose agar or V-8 juice agar to be in a range of 0.25−128 μg/mL by 2-fold serial dilution. Fresh mycelial disks (5 mm in diameter) from actively growing margin colonies of various phytopathogenic fungi on potato dextrose agar or V-8 juice agar were used as inocula after incubation at 20−25 °C for 3−7 days, and the plates were incubated at 20−25 °C for 24−72 h (*26*). The inoculum for yeast was prepared by picking five colonies from 24-h-old cultures of *Candida* species or 48-h-old cultures of *C. neoformans*. The colonies were suspended in 5 mL of sterile 0.85% saline at the turbidity of a 0.5 McFarland standard and diluted to reach 5×10^2 to 2.5×10^3 colony-forming units (CFU)/mL to use as inoculum. CHE-23C was dissolved in DMSO, and the 2-fold serially diluted compound in Sabouraud broth was dispensed into 96-well microtiter plates to be in a range of 0.25−128 μg/mL. An aliquot of inoculum was added to the plates and incubated at 35 °C for 48−72 h. The MIC was defined as the lowest concentration of compound that completely inhibited the growth of the organism when compared to a control plate containing no compound.

Evaluation of in Vivo Antifungal Activity in the Greenhouse. The in vivo antifungal activity of CHE-23C was examined against various diseases such as rice blast (*M. grisea*), rice sheath blight (*Corticium sasaki*), tomato gray mold (*B. cinerea*), tomato late blight (*P. infestans*), wheat leaf rust (*Puccinia recondita*), and barley powdery mildew (*Blumeria graminis* f. sp. *hordei*). Rice (*Oryza sativa* L., cv. Nakdong), tomato (*Lycopersicon esculentum* Mill., cv. Seokwang), cucumber (*Cucumis sativus* L., cv. Hausbackdadagi), wheat (*Triticum aestivum* L., cv. Chokwang), and barley (*Hordeum sativum* Pers. cv. Dongbori) plants were grown in vinyl pots (4.5 cm in diameter) in the greenhouse at 25 ± 5 °C for 1−4 weeks. Seedlings were sprayed until runoff with a 1% Tween 20 solution, which contained CHE-23C dissolved in water and DMSO (99:1, v/v). Control plants were treated with a Tween 20 solution containing 1% DMSO. The treated plant seedling was allowed to stand for 24 h. The in vivo assays of CHE-23C against various plant diseases in the greenhouse were conducted as described previously (*28*), except for the following modifications. For the development of rice blast, the treated rice seedlings of the third-leaf stage were inoculated with *M. grisea* by spraying with a spore suspension (5×10^5 spores/mL) of the fungus. For the rice sheath blight assay, treated rice seedlings at the fourth-leaf stage were inoculated by adding 7-day-old cultures of *C. sasaki* to soil. For tomato gray mold assay, the tomato seedlings of the second-leaf stage were incubated by spraying a spore suspension (10^6 spores/mL) of *B. cinerea*. The inoculated tomato plants were kept in the dark at 20 °C and 100% relative humidity (RH).

Table 3. Antifungal Activities of CHE-23C against Various Pathogens

microorganism	MIC[a] (μg/mL)
Alternaria kikuchiana	8
Botrytis cinerea	8
Colletotrichum lagenarium	8
Fusarium oxysporum	16
Magnaporthe grisea	4
Phytophthora infestans	32
Pythium ultimum	1
Rhizoctonia solani	16
Candida albicans ATCC 10231	64
Candida albicans A207[b]	8
Candida lusitaniae ATCC 6258	128
Candida krusei ATCC 42720	128
Candida tropicalis ATCC 13803	>128[c]
Cryptococcus neoformans ATCC 36556	16

[a] The lowest concentration of compound that completely inhibited the growth of the organism when compared with a control plate containing no compound. The experiment was repeated three times with essentially the same results. [b] *C. albicans* A207; clinical isolate. [c] A value of >128 indicates that the growth of test organism was not inhibited at concentrations up to 128 μg/mL.

Disease severity was evaluated 3 days after inoculation. For tomato late blight assay, the treated tomato seedlings at the second-leaf stage were inoculated with *P. infestans* by spraying with a zoospore suspension (5×10^4 sporangia/mL). After incubation for 1 day at 20 °C and 100% relative humidity, the inoculated seedlings were transferred to growth chamber and kept for 2 days.

The pots were arranged as a randomized complete block with three replicates per treatment. The average value of three estimates for each treatment was converted into percentage fungal control according to the method described previously (*28*): % control = $100 \times [(A - B)/A]$, where A = the area of infection (%) on leaves or sheaths sprayed with Tween 20 solution alone and B = the area of infection (%) on treated leaves or sheaths. The antifungal activities of CHE-23C against various phytopathogenic fungi were compared with those of commercial fungicides such as tricyclazole (rice blast), validamycin (rice sheath blight), fludioxonil (tomato gray mold), dimethomorph and chlorothalonil (tomato late blight), carboxin (wheat leaf rust), and benomyl (barley powdery mildew) as positive controls.

Statistical Analysis. Statistical analysis was conducted with the PROC GLM procedure (SAS Institute, Cary, NC), and the means were determined by the least significant difference test at the P = 0.05 level as described previously (*28*).

RESULTS AND DISCUSSION

Isolation and Purification of Antifungal Compound. A bioactive compound was isolated from the methanol extract of stems and roots of *C. henryi*. The antifungal activities of methanol extract dissolved in DMSO showed inhibitory zone diameters of 11, 31, 25, and 30 mm against *A. kikuchiana*, *M. grisea*, *B. cinerea*, and *P. ultimum*, respectively, on loading of 50 μL of a 10 mg/mL solution into bioassay plates. Thereafter, purification of *C. henryi* was further performed by monitoring antifungal activity against *P. ultimum*, which is one of the difficult pathogens for control and causative agents of damping-off. The compound was purified by solvent fractionation, silica gel, and Sephadex LH-20 column chromatographies, and crystallization produced a white powder. CHE-23C was confirmed as a single peak by analytical HPLC. Moreover, to increase the isolation efficiency, the purification steps of the compound were improved and compared with the yield of a previous method. Therefore, the yield of CHE-23C was increased 6-fold over the procedure described above (**Table 1**). The ^{1}H NMR pattern of CHE-23C isolated from improved method was identical with that of the authentic compound. The bioactive compound was soluble in organic solvents such as

Table 4. In Vivo Disease-Control Activities of CHE-23C Isolated from *Chlorantus henryi* against Various Plant Diseases[a]

compound	concn (μg/mL)	control value[b] (%)					
		RCB	RSB	TGM	TLB	WLR	BPM
CHE-23C	11	67 ± 0.0	50 ± 0.0	7.2 ± 0.0	29 ± 0.0	80 ± 0.0	0.0 ± 0.0
	33	50 ± 0.0	50 ± 18	14 ± 20	91.0 ± 2.0	87 ± 0.0	0.0 ± 0.0
	100	72.0 ± 7.1	69.0 ± 8.8	36 ± 10	93 ± 0.0	100	17 ± 0.0
chlorothalonil[c]	50				94 ± 2.0		
	100				100		
dimethomorph[c]	2				64 ± 10		
	10				100		
tricyclazol[c]	0.5	95 ± 2.4					
	10	100					
validamycin[c]	5		75 ± 0.0				
	50		100				
fludioxonil[c]	5			82 ± 5.1			
	50			100			
carboxin[c]	20					43 ± 14.1	
	50					100	
benomyl[c]	1						90 ± 0.0
	100						100

[a] RCB, rice blast; RSB, rice sheath blight; TGM, tomato gray mold; TLB, tomato late blight; WLR, wheat leaf rust; BPM, barley powdery mildew. [b] The values are expressed as means ± SD of three experiments (P = 0.05). [c] Commercially available chlorothalonil, dimethomorph, tricyclazol, validamycin, fludioxonil, carboxin, and benomyl are used as positive controls against TLB, TLB, RCB, RSB, TGM, WLR, and BPM, respectively.

DMSO, chloroform, and methanol but insoluble in water and *n*-hexane. This compound showed a R_f value of 0.35 in *n*-hexane/ethyl acetate (5:5, v/v) on a silica gel plate (Kieselgel 60 F_{254}, Merck, 0.5 mm). The UV spectrum of the compound in methanol exhibited the absorption maximum at 219 nm. Melting point and $[\alpha]_D^{20}$ of the compound were exhibited at 180−182 °C and −135° (*c* 0.1, DMSO), respectively.

Structure Determination of a Bioactive Compound. The structure of the isolated compound was determined by ESI−mass spectrum and various NMR spectroscopic analyses. The ESI-MS spectral data showing quasi-molecular ion peaks at *m/z* 675.8 $[M - H]^+$ and *m/z* 699.8 $[M + Na]^+$ suggest that the molecular weight might be 676.8 (Figure S1 of the Supporting Information). The ^{13}C NMR spectrum (Figure S2 of the Supporting Information) showed all 38 carbon signals, which were established as 17 quaternary carbons, 9 methine carbons, 6 methylene carbons, and 6 methyl carbons from the results of the DEPT experiment (**Table 2**; Figure S3 of the Supporting Information). The ^{1}H NMR spectrum showed a well-resolved pattern (**Table 2**; Figure S4 of the Supporting Information), and the ^{1}H−^{1}H COSY spectrum (**Figure 1**; Figure S5 of the Supporting Information) indicated the presence of three partial structures of $-CH-CH_2-C{=}C-CH-C{=}C-CH_3$, $-CH-CH_2-C{=}C-CH_2-$, and $CH_3-C{=}CH-CH_3$ in addition to two sets of spin systems for 1,2-disubstituted cyclopropane rings (δ 0.21−0.89−1.88−1.90 and 0.65−1.13−1.37−1.55) (*29*, *30*). Complete assignments of all carbons and protons were confirmed by HMQC spectral analysis (Figure S6 of the Supporting Information). In addition, the presence of singlet methyl proton (δ 2.03, H-g), methyl carbon (δ 20.2, C-g), and carbonyl carbon (δ 169.9, C-f) signals in the ^{1}H and ^{13}C NMR spectra was assigned to be an acetyl group. The positions of the acyl groups were also determined by HMBC experiments (**Table 2**; Figure S7 of the Supporting Information), which showed long-range correlations from nonequivalent methylene protons H-13′ (δ 4.61 and δ 4.81) to two carbonyl carbons C-12′ (δ 170.9) and C-f (δ 169.9). Furthermore, a strong cross peak between nonequivalent methylene protons H-15′ (δ 3.67 and δ 4.10) and carbonyl carbon C-a (δ 167) unambiguously determined the position of a tiglyl residue on C-15′. The stereochemistry was determined by NOESY experiments. NOE interactions were found in those observed for 1,2-disubstituted cyclopropane rings as H-2α/H-2β, H-2α/H-1, H-2α/H-3 and H-2′α/H-2′β, H-2′α/H-1′, H-2′α/H-3′. Additional NOE interaction between H-6′β and H-5′ indicated both to be of β-orientation. Structural analyses with ESI-MS spectrum and various NMR techniques including ^{1}H−^{1}H COSY, HMQC, and HMBC revealed that the isolated compound had the molecular formular $C_{38}H_{44}O_{11}$. Thus, it was identified as dimeric sesquiterpene CHE-23C (**Figure 1**), which we have originally discovered as a novel compound (*31*). Recently, CHE-23C was found to be identical with chloraholide D, having potassium channel blocking activity (*32*).

In Vitro and in Vivo Antifungal Activities of CHE-23C. The antifungal activities of the isolated CHE-23C were determined by MIC using the agar dilution method and a slight modification of the CLSI method against phytopathogenic fungi and human pathogenic fungi, respectively. CHE-23C showed considerable antifungal activities against various phytopathogenic fungi such as *A. kikuchiana*, *B. cinerea*, *C. lagenarium*, *M. grisea*, and *P. ultimum* with MIC values of 1−16 μg/mL (**Table 3**). Among them, the compound exhibited the most potent antifungal activity against *P. ultimum* at the concentration of 1 μg/mL. In addition, the compound showed inhibitory activity against *C. albicans* A207 and *C. neoformans* at concentrations of 8 and 16 μg/mL, respectively, whereas it exhibited weak antifungal activities against other *Candida* strains.

In vivo protective activities against rice blast, rice sheath blight, tomato gray mold, tomato late blight, wheat leaf rust, and barley powdery mildew were evaluated under the greenhouse condition. The disease-control efficacy of CHE-23C against various plant diseases was 69−100% at the concentration of 100 μg/mL except for tomato gray mold and barley powdery mildew (**Table 4**). Especially, the compound exhibited 91% protective activity against *P. infestans*, which is a difficult pathogen to control and the causative agent of tomato late blight, at the concentration of 33 μg/mL. Disease-control activity of the compound was stronger than that of commercially available chlorothalonil, which is used as a positive control of tomato late blight, although its activity was weaker than that of dimethomorph. In addition, CHE-23C represented 80 and 87% control efficacy against wheat leaf rust (*P. recondita*) at concentrations of 11 and 33 μg/mL, respectively. In particular, the compound at a concentration of 11 μg/mL was more effective in vivo against wheat leaf rust than the commercial fungicide carboxin.

Although a number of sesquiterpenoids and sesquiterpenoid oligomers isolated from the genus *Chloranthus* have biological activities such as cell adhesion inhibition (*33*), hepatoprotection (*34*), potassium channel inhibition (*32*, *35*), and bacterial growth inhibition (*36*), only limited information is available for the sesquiterpenoids and diterpenoids from *C. henryi*. In particular, there is little information about in vitro and in vivo antifungal activities of sesquiterpene against phytopathogenic fungi. Taken together, this is the first report that the dimeric sesquiterpenoid CHE-23C showed potent protective activities in vivo against *P. infestans* and *P. recondita*. Therefore, CHE-23C may be a lead compound for the development of agrochemical agents. The mode of action of this compound against plant pathogens remains to be investigated.

Supporting Information Available: ESI-MS, ^{13}C NMR, DEPT, ^{1}H NMR, COSY, HMQC, and HMBC spectra for structural identification of CHE-23C.This material is available free of charge via the Internet at http://pubs.acs.org.

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Received February 27, 2009. Revised manuscript received May 14, 2009. Accepted May 18, 2009. This work was supported by grants from the Plant Diversity Research Center of 21st Century Frontier Research Program funded by the Ministry of Education, Science and Technology and Biogreen21, Rural Development Administration, Republic of Korea.