

Sesquiterpenes from an Eastern African Medicinal Mushroom Belonging to the Genus *Sanghuangporus*

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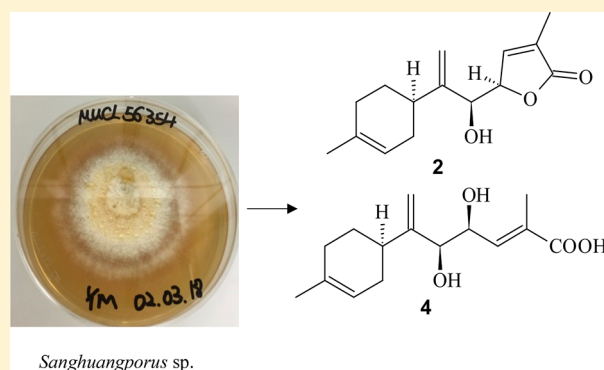
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Supporting Information

ABSTRACT: During the course of searching for new anti-infective and other biologically active secondary metabolites from Kenyan basidiomycetes, 13 previously undescribed metabolites, (6R,7S,10R)-7,10-epoxy-7,11-dimethyldodec-1-ene-6,11-diol (**1**) and 12 sesquiterpenes named elgonenes A–L (**2**–**13**), and the known compound *P*-coumaric acid (**14**) were isolated from a basidiomycete collected in Mount Elgon Natural Reserve. The producing organism represents a new species of the genus *Sanghuangporus*, which is one of the segregates of the important traditional Asian medicinal mushrooms that were formerly known as the “*Inonotus linteus*” complex. The structure elucidation of compounds **1**–**13**, based on 2D NMR spectroscopy, high-resolution mass spectrometry, and other spectral methods, and their antibacterial, antifungal, and cytotoxic activities are reported.



The phylum Basidiomycota comprises the mushroom-forming fungi and various other organisms that represent a considerable part of the global biodiversity. Recent molecular ecology studies have revealed huge diversity of fungi in different habitats, including soil, plants, and invertebrates.¹ Unfortunately, most of these fungi and especially the majority of the species from tropical areas have neither been cultured nor studied for potential beneficial traits such as the production of antibiotics or other useful secondary metabolites.² For the last four years, we have embarked on an extensive study of the secondary metabolites of basidiomycetes collected from Kenya's tropical rain forests, Kakamega and Mount Elgon National Reserves. This study has resulted in the discovery of novel structurally diverse metabolites.^{3–8} The genus *Sanghuangporus* in the family Hymenochaetaceae and the order Hymenochaetales belongs to the group of medicinal mushrooms commonly known as the *Inonotus linteus* complex (also referred to as the *Phellinus linteus* complex). This complex consists of species originally described in the genera *Phellinus* and *Inonotus*, which were eventually transferred to *Inonotus* based on molecular studies.⁹ Although the species of the *I. linteus* complex were placed in the genus *Inonotus*, their perennial basidiomes with a dimitic hyphal system distinguish the complex from other species in the genus. This has led to the segregation of this group to accommodate the *I. linteus* complex in the new genera *Sanghuangporus* and *Tropicoporus*.¹⁰ These medicinal mushrooms have been used as folk medicines in Asian countries for thousands of years, in particular for the

treatment of hemorrhage, hemostasis, and diseases related to menstruation. The species *Phellinus linteus* (*Tropicoporus linteus*) in particular has been used in traditional Chinese medicine because of its therapeutic effects on various ailments including tumor, diabetes, inflammation, and obesity.^{9,11}

The novel phelligridin L together with the four known sesquiterpenes ionylidenecacetic acid, 1S-(2E)-5-[(1R)-2,2-dimethyl-6-methylidenecyclohexyl]-3-methylpent-2-enoic acid, and phellidines D and E were reported previously from a study of *Sanghuangporus* sp. (MUCL55592).⁵ In the study reported here a fungal strain identified as *Sanghuangporus* sp. (MUCL56354) was examined for the production of bioactive metabolites. Herein, we describe the isolation, structure elucidation, and biological evaluation of 13 previously undescribed secondary metabolites (**1**–**13**) and *P*-coumaric acid (**14**).

RESULTS AND DISCUSSION

The strain MUCL 56354 was identified by comparison of morphological characteristics and sequencing of the 5.8S/ITS nrDNA,¹² as described in the [Experimental Section](#). A BLAST search in GenBank confirmed the generic affinities of the strain to the genus *Sanghuangporus* in the Hymenochaetaceae by a closest hit to KP030787.1 (*Sanghuangporus microcystideus*) with 96% identity (cf. Figure 109 in the [Supporting](#)

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Chart 1

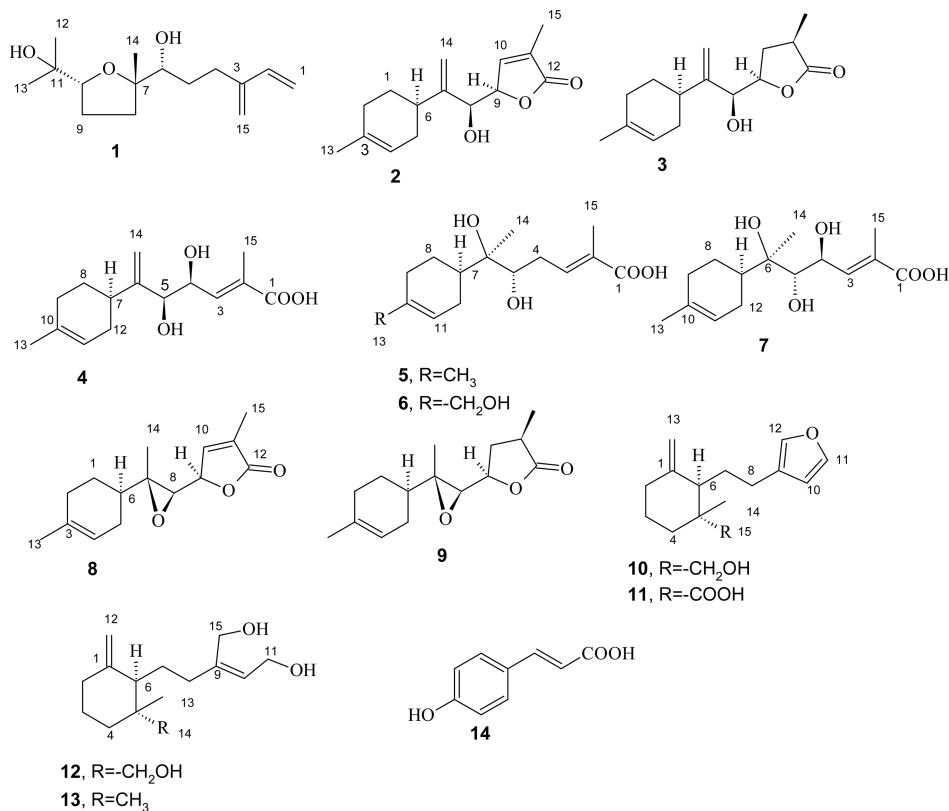


Table 1. NMR Spectroscopic Data for Compound 1 (¹H 700 MHz, ¹³C 175 MHz in Acetone-*d*₆), Compounds 2 and 3 (¹H 700 MHz, ¹³C 175 MHz, in CDCl₃), and Compound 4 (¹H 500 MHz, ¹³C 125 MHz, in CDCl₃)

no.	1		2		3		4	
	¹³ C	¹ H/HSQC	¹³ C	¹ H/HSQC	¹³ C	¹ H/HSQC	¹³ C/DEPT	¹ H/HSQC
1	113.8, CH ₂	5.05, dd (17.64, 1.08) 5.30, dd (17.64, 1.08)	29.4, CH ₂	1.55, m 1.88, m	31.5, CH ₂	2.02, m 2.06, m	170.6, C	
2	139.9, CH	6.39, dd (17.64, 10.76)	30.6, CH ₂	1.99, m 2.07, m	30.6, CH ₂	1.98, m 2.05, m	130.3, C	
3	147.8, C		133.9, C		133.9, C		140.1, CH	6.87, br d (8.82)
4	29.2, CH ₂	a: 2.23, m b: 2.55, m	120.2, CH	5.41, m	120.2, CH	5.40, m	69.9, CH	4.53, dd (8.82, 4.52)
5	31.9, CH ₂	a: 1.42, m b: 1.69, m	31.6, CH ₂	2.05, m 2.12, m	29.4, CH ₂	1.49, m 1.86, m	76.4, CH	4.34, d (4.52)
6	77.2, CH	3.50 dd (17.64)	36.9, CH	2.14, m	36.9, CH	2.05, m	152.7, C	
7	86.9, C		152.1, C		151.1, C		36.8, CH	2.12, m ^a
8	32.7, CH ₂	a: 1.50, m b: 2.12, m	73.3, CH	4.41, d (4.30)	71.9, CH	4.54, br s	29.6, CH ₂	1.49, m 1.84, m
9	27.3, CH ₂	1.87, m 1.97, m	82.3, CH	4.95, m	78.9, CH	4.49, ddd (3.44, 6.29, 9.84)	30.6, CH ₂	1.98, m ^a 2.04, m ^a
10	85.5, CH	3.79, t (7.42)	145.3, CH	7.07, m	29.4, CH ₂	1.95, m 2.20, m	133.7, C	
11	71.7, C		131.7, C		35.5, CH	2.68, m	120.5, CH	5.41, m
12	26.6, CH ₃	1.07, s	173.9, C		179.4, C		31.6, CH ₂	^a 1.99, m ^a 2.08, m
13	27.8, CH ₃	1.20, s	23.4, CH ₃	1.67, s	23.4, CH ₃	1.66, s	23.4, CH ₃	1.66, s
14	23.7, CH ₃	1.11, s	111.4, CH ₂	5.15, br s 5.27, br s	110.8, CH ₂	5.07, s 5.28, s	111.3, CH ₂	5.07, s 5.22, s
15	116.2, CH ₂	a: 5.04, s b: 5.05, s	10.9, CH ₃	1.96, t (1.94)	15.1, CH ₃	1.30, s	13.0, CH ₃	1.92, s

^aPeaks overlapping with other peaks.

Table 2. NMR Spectroscopic Data for Compound 5 (^1H 500 MHz, ^{13}C 125 MHz in Acetone- d_6), Compound 6, (^1H 500 MHz, ^{13}C 125 MHz in Methanol- d_4), and Compounds 7–9 (^1H 700 MHz, ^{13}C 175 MHz in CDCl_3)

no.	5		6		7		8		9	
	$^{13}\text{C}/$ DEPT	$^1\text{H}/\text{HSQC}$	$^{13}\text{C}/$ DEPT	$^1\text{H}/$ HSQC	$^{13}\text{C}/$ DEPT	$^1\text{H}/\text{HSQC}$	^{13}C	$^1\text{H}/\text{HSQC}$	^{13}C	$^1\text{H}/\text{HSQC}$
1	169.2, C		171.8, C		171.0, C		24.6, CH_2	1.48, m 1.88, m	24.5, CH_2	1.48, m 1.86, m
2	128.9, C		129.9, C		130.3, C		29.8, CH_2	1.98, m	29.9, CH_2	1.98, m
3	142.4, CH	6.99, m	142.7, CH	6.98, m	141.5, CH	6.89, dd (8.93, 1.18)	134.1, C		134.1, C	
4	31.8, CH_2	2.32, m 2.59, m	32.0, CH_2	2.31, m 2.58, m	69.9, CH	4.73, dd (8.82, 6.45)	119.5, CH	5.35, m	119.6, CH	5.35, m
5	75.2, CH	3.68, dd (2.59, 9.92)	75.6, CH	3.64, m	74.9, CH	3.72, d (6.45)	27.0, CH_2	1.86, m 2.00, m	27.1, CH_2	1.86, m 2.05, m
6	75.6, C		76.7, C		77.4, C		41.4, CH	1.40, m	41.6, CH	1.40, m
7	41.3, CH	1.77, m	42.3, CH	1.79, m	40.7, CH	1.74, m	64.2, C		64.1, C	
8	24.6, CH_2	1.34, m 1.81, m	27.7, CH_2	1.36, m ^a 1.84, m ^a	23.5, CH_2	1.35, m 1.81, m	61.1, CH	2.57, d (8.39)	63.1, CH	2.80, d (7.96)
9	31.7, CH_2	1.93, m	27.7, CH_2	2.04, m ^a 2.13, m ^a	30.6, CH_2	1.98, m ^a 2.04, m ^a	78.1, CH	4.63, dt (1.77, 8.50)	75.2, CH	4.17, ddd (5.83, 7.96, 13.55)
10	133.9, C		138.5, C		133.8, C		147.1, CH	7.22, m	35.5, CH_2	1.88, m 2.64, tt (5.81, 8.60)
11	122.3, CH	5.38, m	123.8, CH	5.67, m	120.5, CH	5.40, m	130.9, C		35.0, CH	2.69, m
12	26.7, CH_2	2.01, m ^a 2.16, m ^a	26.7, CH_2	2.04, m ^a 2.20, m ^a	25.6, CH_2	2.05, m ^a 2.09, m ^a	173.6, C		179.1, C	
13	23.6, CH_3	1.61, br s	67.4, CH_2	3.91, s	23.3, CH_3	1.66, s	23.4, CH_3	1.64, br s	23.4, CH_3	1.64, br s
14	19.8, CH_3	1.16, s	19.2, CH_3	1.17, s	19.8, CH_3	1.31, s	13.9, CH_3	1.38, s	13.9, CH_3	1.31, s
15	12.8, CH_3	1.81, d (1.22)	12.9, CH_3	1.84, s	13.0, CH_3	1.96, s	10.8, CH_3	1.97, d (1.72)	15.2, CH_3	1.33, d (6.88)

^aPeaks overlapping with other peaks.

Information). Further taxonomic studies including comparisons with type specimens of related species are ongoing to characterize this strain to the species level, but it constitutes an undescribed taxon according to the data presently available.

The extracts of the strain (MUCL56354) exhibited activity against *Bacillus subtilis* with an MIC 37.5 $\mu\text{g}/\text{mL}$ (compared to the positive control ciprofloxacin at MIC 3.1 $\mu\text{g}/\text{mL}$). Extensive chromatography of the extracts led to the bioactivity-guided isolation of 13 previously undescribed compounds and the known *P*-coumaric acid, the latter of which was identified by comparing its NMR data with those reported in the literature.¹³

Compound **1** was obtained from the supernatant as a yellow oil with the molecular formula $\text{C}_{15}\text{H}_{26}\text{O}_3$ deduced from the HR-ESIMS data. The ^{13}C NMR spectroscopic data of **1** revealed the presence of 15 carbon signals, which were further identified as three methyl, six methylene, three methine, and three nonprotonated carbons (one sp^2 carbon and two sp^3 oxygenated carbons) from the DEPT NMR data. The ^1H NMR spectrum revealed three methyl singlets resonating at δ 1.07 (H_3 -12), 1.11 (H_3 -14), and 1.20 (H_3 -13). A doublet and triplet resonating at δ 3.50 and 3.79 attributed to oxygenated methines were also recorded. HMBC correlations of H_2 -1 to C-2/C-3, H_2 -15 to C-2/C-4, and H_2 -5 to C-3/C-4/C-6/C-7 along with the COSY correlations of H_2 -1 to H-2 and H_2 -5 to H_2 -4/H-6 confirmed the first side chain in the molecule attached at C-7. The methyl protons H_3 -14 showed HMBC correlations to C-6/C-7/C-8, while methylene protons H_2 -9 correlated with C-7/C-8/C-10/C-11. Cross-peaks were recorded between H_2 -8 and H_2 -9 in the COSY spectra. The chemical shifts of C-7 (δ 86.9) and C-10 (δ 85.5) and the HMBC correlation of H-10 to C-7 indicated that these carbons

were connected via an oxygen bridge forming the oxolane. The methyl group protons H_3 -12 showed HMBC correlations to C-11/C-10/C-13. Based on the molecular formula of **1** and the chemical shift of C-11 (δ 71.7), it was determined that a hydroxy group was attached to this carbon. Cross-peaks were also observed in the ROESY spectra between H_3 -14 and H-6/ H_b -5/ H_a -8, H-6 and H_b -5/ H_a -4/ H_a -8, and H_a -9 and H_a -8/H-10. The closely related compounds neroplofurol and 7,10-epoxy-3,7,11-trimethyldodec-2-ene-1,6,11-triol have been reported from the plant *Oplopanax horridus* and the actinobacterium *Streptomyces scopuliridis*, respectively.^{14,15} The relative stereochemistry at C-10 for this class of compounds was reported to be significantly impacted by the orientation of 14-Me and the 2-hydroxysopropyl moiety. According to Inui et al.,¹⁴ a chemical shift of C-10 (about δ 85 ppm) indicates the *anti*-orientation of these two substituents because of the γ -gauche effect caused by an alkyl substituent at C-6. For the case where the two substituents are in the *syn*-orientation, the C-10 is shifted downfield by around 3.3 ppm. Based on these literature data and the ROESY correlations observed by us, the relative stereochemistry of **1** was assigned as 6R*, 7S*, 10R*. The absolute stereochemistry of **1** was consequently assigned by derivatization with both R- and S-MTPA chloride to their corresponding S- and R-MTPA esters.¹⁶ The C-5/C-4 methylene protons gave positive values, while the H_3 -14/ H_2 -8/ H_2 -9 gave negative values for the $\Delta\delta_{\text{SR}} = \delta(\text{S-MTPA ester}) - \delta(\text{R-MTPA ester})$ (see Table 1 in the Supporting Information). Therefore, the absolute stereochemistry at C-6 was assigned as R and the absolute configurations of the other stereocenters were assigned as 7S and 10R. The structure of **1** was concluded to be (6R,7S,10R)-7,10-epoxy-7,11-dimethyldodec-1-ene-6,11-diol (**1**).

Table 3. NMR Spectroscopic Data for Compound 10 (^1H 500 MHz, ^{13}C 125 MHz in Acetone- d_6), Compound 11 (^1H 700 MHz, ^{13}C 175 MHz in Acetone- d_6), Compound 12 (^1H 500 MHz, ^{13}C 125 MHz in CDCl_3), and Compound 13 (^1H 700 MHz, ^{13}C 175 MHz in CDCl_3)

no.	10		11		12		13	
	$^{13}\text{C}/\text{DEPT}$	$^1\text{H}/\text{HSQC}$	$^{13}\text{C}/\text{DEPT}$	$^1\text{H}/\text{HSQC}$	$^{13}\text{C}/\text{DEPT}$	$^1\text{H}/\text{HSQC}$	$^{13}\text{C}/\text{DEPT}$	$^1\text{H}/\text{HSQC}$
1	150.1, C		148.8, C		150.1, C		149.1, C	
2	33.0, CH_2	2.03, m ^a 2.16, m	34.5, CH_2	2.03, m 2.25, m ^a	33.0, CH_2	2.03, m 2.16, m ^a	32.3, CH_2	2.03, m 2.06, m
3	24.2, CH_2	1.53, m ^a	25.2, CH_2	1.59, m ^a 1.66, m	23.6, CH_2	1.56, m ^a	23.6, CH_2	1.53, m ^a
4	31.7, CH_2	1.34, m ^a 1.59, m	34.8, CH_2	1.56 ^a 1.95, td (4.62, 8.44)	33.1, CH_2	1.29, m ^a ^a 1.63, m	36.1, CH_2	1.23, m ^a 1.47, m ^a
5	40.1, C		49.3, C		40.0, C		34.9, C	
6	49.3, CH	2.06, m ^a	49.1, CH	2.66, dd (2.37, 11.4)	45.5, CH	2.12, dd (2.90, 11.75)	53.8, CH	1.73, dd (3.01, 11.19)
7	27.2, CH_2	1.68, m 1.75, m	28.4, CH_2	1.58, m ^a 1.75, m	22.9, CH_2	1.63, m ^a	24.8, CH_2	1.50, m ^a 1.63, m
8	23.9, CH_2	2.21, m ^a 2.40, m	23.9, CH_2	2.27, m ^a 2.47, m	34.4, CH_2	2.03, m 2.16, m ^a	34.4, CH_2	1.90, m 2.13, m
9	126.4, C		126.1, C		143.6, C		144.5, C	
10	111.9, CH	6.35, br s	111.9, CH	6.34, br s	127.9, CH	5.62, t (6.87)	126.1, CH	5.64, t (6.88)
11	143.7, CH	7.44, br s	143.7, CH	7.43, br s	58.5, CH_2	4.14, dd (12.36, 6.87) 4.22, m ^a	58.7, CH_2	4.24, br d (6.88) 4.28, dd (3.44, 6.88) ^a
12	139.8, CH	7.34, br s	139.8, CH	7.34, br s	108.8, CH_2	4.59, s 4.86, s	109.2, CH_2	4.56, s 4.78, s
13	109.8, CH_2	4.60, s 4.81, s	109.8, CH_2	4.69, s 4.87, s	19.1, CH_3	0.74, s	26.4, CH_3	0.85, s
14	21.6, CH_3	0.82, s	19.8, CH_3	1.06, s	69.5, CH_2	3.29, d (11.29) 3.56, d (11.29)	28.4, CH_3	0.92, s
15	68.6, CH_2	3.23, d (10.76) 3.50, d (10.76)	178.5, C		60.7, CH_2	4.05, s 4.23, s	61.2, CH_2	4.18, s 4.19, s

^aPeaks overlapping with other peaks.

Compound **2** was isolated as a yellow oil from both the supernatant and the mycelia. The HR-ESIMS data revealed the molecular formula to be $\text{C}_{15}\text{H}_{20}\text{O}_3$ with six degrees of unsaturation. Two methyl peaks at δ 1.67 (H_3 -13) and 1.96 (H_3 -15) and two olefinic singlets at δ 5.15 and 5.27 were recorded in the ^1H NMR spectrum. A total of 15 carbons, two methyl groups, four methylene groups, five methine groups, and four nonprotonated carbons could be identified from the ^{13}C and DEPT NMR data. In the HMBC spectrum correlations of H-9 to C-10/C-11/C-12/C revealed the dihydrofuran part of the molecule. Correlations of H_3 -15 to C-10/C-11/C-12 were also observed in the HMBC spectra. Further, HMBC correlations of H-8 to C-6/C-7/C-9/C-10/C-14 were observed. Cross-peaks in the COSY spectrum were also recorded between H-9 and H-8/H-10. The position of the vinyl group was confirmed from the HMBC correlations of H_2 -14 to C-6/C-7/C-8. The COSY correlations of H_2 -1 to H-6/ H_2 -2 and H_2 -5 to H-6/H-4 along with the HMBC correlations of H_3 -13 to C-2/C-3/C-4 confirmed the cyclohexyl part of the molecule connected through C-6. ROESY correlations were observed between H-8 and H-6/H-9. The absolute stereochemistry of **2** was assigned by derivatization with R and S MTPA chloride. The $\Delta\delta_{\text{SR}} = \delta(\text{S-MTPA ester}) - \delta(\text{R-MTPA ester})$ for protons neighboring C-8 (see Table 2 in the Supporting Information) led to the S-configuration assignment at C-8 and consequently the assignment of 6R and 9R for the other respective stereocenters.

Compound **3**, with the molecular formula $\text{C}_{15}\text{H}_{22}\text{O}_3$ and five degrees of unsaturation deduced from HR-ESIMS data, was isolated from both the supernatant and the mycelial extracts. Analysis of the 1D and 2D NMR data for **3** revealed a similar structure to **2** with the double bond at C-10 missing in compound **3**. Analysis of the ^{13}C NMR data for **3** indicated the absence of the olefinic C-10 occurring at δ 145.3 and the C-11 (131.7) in compound **2** and a methylene and methine signals at δ 29.4 and 35.5, respectively, were observed. The H_3 -15 (δ 1.30) showed HMBC correlation to C-10 (δ 29.4)/C-11 (δ 35.5)/C-12 (δ 179.4). Additional ROESY correlations between H-9 and H-11 were also observed. Since compounds **2** and **3** were obtained as congeners, the absolute stereochemistry of **3** was assigned as 6R, 8S, 9R, and 11R. Compounds **2** and **3** are structurally related to phelilane D from *Inonotus vaninii*,¹⁷ i.e., a species that is closely related to *Sanghuangporus*.

Compound **4** was isolated as a yellow oil only from the supernatant. The HR-ESIMS data revealed the molecular formula to be $\text{C}_{15}\text{H}_{22}\text{O}_4$ with five degrees of unsaturation. Analysis of the 1D and 2D NMR data suggested a structure closely related to compound **2**. Similar COSY, HMBC, and ROESY correlations were observed except for the HMBC correlations of H-4 to C-1, which was missing in **4**, indicating that this compound had an open end chain instead of the dihydrofuran in **2**. This was in agreement with the HR-ESIMS data, which showed the presence of four oxygen atoms for this molecule. The olefinic double bond between C-2 and C-3 was

assigned an *E*-configuration because of the upfield shifts of C-15 (δ 13.0). ROESY correlations of H-7 suggested that this compound shared the same *R*-configuration with **2** at C-7; hence the stereochemistry of this molecule was assigned as 4*S*, 5*S*, and 7*R*. This compound is closely related to 2-methyl-6-(4-methyl-3-cyclohexen-1-yl)-2,6-heptadienoic acid, (*E*)-(9*CI*), previously reported in the patent by Kobayashi et al. *Tropicoporus linteus*.¹⁸

Compound **5** was isolated as a yellow oil from the supernatant extract. It had the molecular formula $C_{15}H_{24}O_4$ and showed four degrees of unsaturation as established from the HR-ESIMS data. Three methyl singlets at δ 1.16 (H₃-14), 1.61 (H₃-13), and 1.81 (H₃-15) were observed in the ¹H NMR spectrum. Further, three methyl, four methylene, four methane, and four nonprotonated carbons (three *sp*² carbons and one oxygenated *sp*³ carbon) were identified from the ¹³C and DEPT NMR data. HMBC correlations of H₃-15 to C-1/C-2/C-3, H-5 to C-3/C-4/C-6/C-14, and H₃-14 to C-5/C-6/C-7 established the structure of the side chain. COSY correlations were also observed between H-4 and H-3/H-5. Similar COSY and HMBC correlations making up the cyclohexyl moiety connected to the side chain through C-7 in compounds **1**, **2**, and **3** were also established for this compound. The double bond at C-2 was assigned as *E* based on the upfield shift of C-15 (δ 12.8). ROESY correlations H-14 to H-7/H₂-4 (δ 2.32)/H₂-12 (δ 2.01) indicated that these protons were on the same side of the plane. On the other hand cross-peaks were observed between H-5 and H₂-4 (δ 2.59)/H₂-12 (δ 2.16) in the ROESY spectra. To assign the absolute stereochemistry, both *S*- and *R*-MTPA ester derivatives of **5** were prepared from *R*- and *S*-MTPA chloride, respectively. The $\Delta\delta_{SR} = \delta(S\text{-MTPA ester}) - \delta(R\text{-MTPA ester})$ for protons neighboring C-5 gave negative values for the C-4 methylene protons (−0.005 and −0.008) and positive values for H₃-14 and H-7, i.e., +0.003 and +0.012, respectively (Table 3 in the SI). Therefore, the absolute stereochemistry at C-5 was assigned as *S*, and consequently the other centers were assigned as 6*R*, 7*R*. Phellilane H, previously reported from *Phellinus linteus* (current valid name *Tropicoporus linteus*¹⁰), has a similar structure to **5** but a different stereochemistry.¹⁸

Compound **6**, with the molecular formula $C_{15}H_{24}O_5$ and four degrees of unsaturation deduced from HR-ESIMS data, was isolated from supernatant extracts. Analysis of the 1D and 2D NMR data for **6** indicated a similar structure to **5** with the C-13 methyl group missing in compound **6**, but an oxymethylene peak at δ 67.4 (C-13) was recorded instead. The H₂-13 (δ 3.91) showed HMBC correlations to C-9/C-10/C-11.

Compound **7**, which was isolated as a yellow oil only from the supernatant extract, had a molecular formula of $C_{15}H_{24}O_5$ and four degrees of unsaturation established from the HR-ESIMS data. Analysis of the 1D and 2D NMR data for **7** indicated a similar structure to that of **5** with the C-4 methylene group replaced by an oxymethine signal resonating at δ 69.9. COSY correlations between the oxymethine proton (δ 4.73) and H-3/H-5 were observed. Furthermore, a correlation of this proton (H-4) to C-2/C-3/C-5/C-6 could be established in the HMBC spectra. In the ROESY spectra of **7**, similar correlations to those observed for **5** were recorded. In addition, cross-peaks in the ROESY spectra of **7** were recorded between H-4 and H₃-14/H-7. Thus, the 4*S*, 5*S*, 6*R*, and 7*R* configurations were concluded for this molecule. The comparison of the circular dichroism (CD) spectra of **5** and

7 supported this conclusion, as the electronic circular dichroism (ECD) curves of these compounds were comparable in the range of 204–240 nm (positive Cotton effect registered) (Figure 1). Compounds **6** and **7** are also closely related to phellilane H.¹⁸

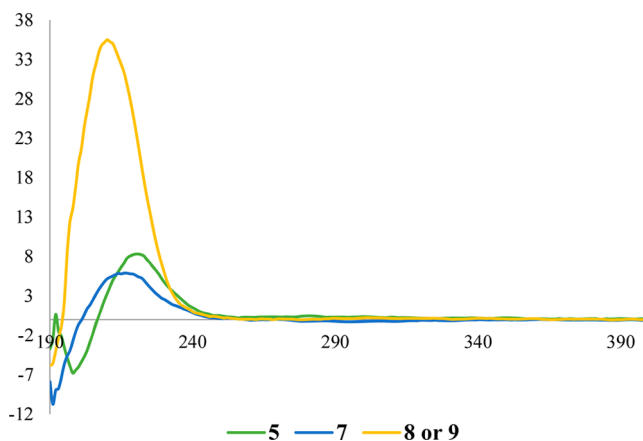


Figure 1. ECD spectra of compounds **5**, **7**, and **8/9** in ethanol.

Compounds **8** and **9** were isolated as yellow oils from both the supernatant and mycelial extracts. The mixture of the two compounds (ratio 2:1) could not be separated, but their NMR data were clearly resolved and, thus, their structures could be determined independently. HR-ESIMS data revealed their molecular weights as $C_{15}H_{20}O_3$ and $C_{15}H_{22}O_3$ for compounds **8** and **9**, respectively.

For compound **8** HMBC correlations of H₃-15 to C-10/C-11/C-12, H-9 to C-7/C-8/C-10/C-11/C-12, and H₃-14 to C-6/C-7/C-8 together with the COSY correlations of H-9 to H-8/H-10 confirmed one part of the structure. The chemical shift of C-9 (δ 78.1) and the HMBC correlations of H-9 to C-12 suggested that C-9 and C-12 were linked via an oxygen bridge, forming a dihydrofuran. Similar HMBC and COSY correlations making up the cyclohexyl part of the molecules already described above for the congeners were also recorded for this molecule. Based on the chemical shifts of the C-7 (δ 64.2) and C-8 (δ 61.1) an epoxide was concluded to be in the molecule.

The 1D and 2D NMR data of **9** were similar to those of **8**, with the difference being the absence of the olefinic bond between C-10 and C-11. For both compounds ROESY correlations of H₃-14 to H-6/H-8/H-9 and H-8 to H-6/H-9 were observed and cross-peaks were recorded between H-9 and H-11 in the ROESY spectra of **9**. These correlations revealed that these compounds had the same stereochemistry as **5** at C-5 and C-6. The stereochemistry of this molecule was thus assigned as 6*R*, 7*R*, 8*R*, and 9*R*. Comparison of the CD spectra of the mixture of compounds **8/9** with that of **5** supported this assignment, as only a positive Cotton effect in the range of 194–240 nm was observed for the mixture (Figure 1). Therefore, the structures of **8** and **9** were unambiguously established as (9*R*)-11-methyl-9-[(7*R*,8*R*)-7-methyl-6-[(6*R*)-3-methylcyclohex-3-en-6-yl]oxiran-8-yl]furan-12-one and (9*R*,11*R*)-11-methyl-9-[(7*R*,8*R*)-7-methyl-6-[(6*R*)-3-methylcyclohex-3-en-6-yl]oxiran-8-yl]oxolan-12-one, respectively. These two compounds are closely related to phellilane E from *Tropicoporus linteus*.¹⁸

Compound **10**, with the molecular formula $C_{15}H_{22}O_2$ and five degrees of unsaturation determined from the HR-ESIMS

data, was obtained as a yellow oil only from the supernatant extract. One methyl, seven methylene, four methane, and three nonprotonated carbons (two sp^2 carbons and one sp^3 carbon) were identified in the ^{13}C and DEPT NMR spectra. COSY correlations of H₂-3 to H₂-2/H₂-4, as well as the HMBC correlations of H₂-13 to C-1/C-2/C-6 and H₃-14/H₂-15 to C-4/C-5/C-6, confirmed the cyclohexane ring part in the molecule. Further cross-peaks in the COSY spectra between H₂-7 and H-6/H₂-8 were established. In the HMBC spectra H₂-8 showed correlations to C-6/C-7/C-9/C-10/C-12, H-10 to C-9/C-11/C-12, and H-12 to C-9/C-10/C-11. Based on the chemical shift of C-11 (δ 143.7) and C-12 (δ 139.8) the two carbons were linked via an oxygen bridge forming the furan. Hence the structure was elucidated as {5-[9-(furan-9-yl)ethyl]-5-methyl-1-methylidenecyclohexyl}methanol.

Compound **11** was isolated as a yellow oil from both the supernatant and mycelia extracts. The HR-ESIMS data revealed the molecular formula to be C₁₅H₂₀O₃ with 6 degrees of unsaturation. The 1D and 2D NMR data for **11** revealed a similar structure to **10** except that the oxymethylene group at δ 68.6 (C-15) in **10** was missing and a carboxylic acid peak at δ 178.5 (H-15) was observed instead. The H₃-14 correlated to this carbon in the HMBC spectra. Interestingly, compounds **10** and **11** are somewhat structurally related to dihydropallescensin-2 from the defensive organs of the nudibranch *Tyrinna nobilis*.¹⁹

Compound **12** was isolated as a yellow oil from the supernatant with the molecular formula C₁₅H₂₆O₃ established from the HR-ESIMS data. 1D and 2D NMR data for **12** indicated a structure similar to **10** with the open end chains instead of the furan. Two oxymethylene carbons at δ 58.5 (C-11) and 60.7 (C-15) were identified in the ^{13}C /DEPT NMR data. HMBC correlations of the diastereotopic protons H₂-11 (δ 4.14, 4.22) to C-9/C-10 and H₂-15 (δ 4.05, 4.23) to C-8/C-9/C-10 were observed. The olefinic bond between C-9 and C-10 was assigned the Z-configuration because of the ROESY correlations of H-10 (δ 5.62) to the diastereotopic protons H₂-8 (δ 2.03, 2.16). Therefore, the structure was unambiguously determined to be (9Z)-9-{9-[(5-(hydroxymethyl)-5-methyl-1-methylidenecyclohexyl)ethyl]}but-9-ene-11,15-diol.

Compound **13** was obtained as a yellow oil from both supernatant and mycelia extracts with the molecular formula C₁₅H₂₆O₂ determined from the HR-ESIMS data. The 1D and 2D NMR data indicated a similar structure to that of compound **12** with the difference being the substituents at C-5. The C-14 hydroxymethyl group in **12** was replaced with a methyl group (δ 28.4). The structure of **13** was therefore concluded to be (9Z)-9-{9-[(5,5-dimethyl-1-methylidenecyclohexyl)ethyl]}but-9-ene-11,15-diol. The absolute stereochemistry of **13** at C-6 was assigned as S from comparison of its optical rotation ($[\alpha]_D^{20}$ +7.9 in methanol) to that of the known compound dihydropallescensin-2 ($[\alpha]_D^{20}$ +6.0 in chloroform), which has the same stereocenter.²⁰ To determine the stereochemistry at C-6 for compounds **10** and **11**, CD spectra of these three compounds were measured. While a negative Cotton effect was observed in the range of 190–220 nm of the ECD curve of compound **13** in EtOH, a positive Cotton effect was recorded for **10** and **11** (opposite that of **13**) in the same region, suggesting a 6R-configuration for these two compounds (Figure 2). H₃-14 and H-6 did not correlate in the ROESY spectra, and therefore, a 5R-configuration was concluded for these compounds. Com-

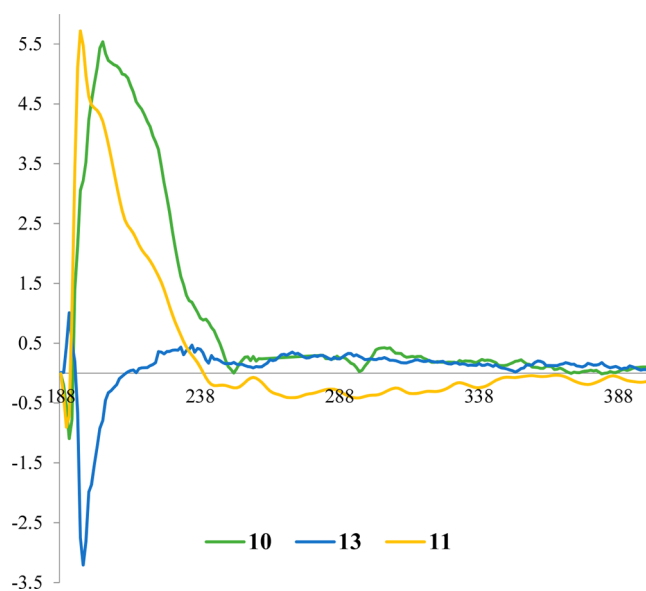


Figure 2. ECD spectra of compounds **10**, **11**, and **13** in ethanol.

pounds **12** and **13** are closely related to *trans*- γ -monocyclofarnesol.²¹

As already mentioned above, some closely related compounds named phelilanes have previously been patented from *Tropicoporus linteus* as part of the composition of an antibacterial agent that is being marketed for the treatment of ailments as diverse as gastroenteric dysfunction, diarrhea, hemorrhage, cancers, and prevention of periodontal disease and in the form of toothpaste and mouthwash agents in Asia.¹⁸ These compounds have to the best of our knowledge never been published in peer-reviewed papers, but a comparison of their spectral data proved helpful.

Compounds **4**, **5**, and **7–14** were evaluated for their antimicrobial activity (Table 4). Metabolites **1–3** and **6** were not tested because they were produced in small quantities (0.42, 0.28, 0.49, and 0.89 mg for each) and they were used in the pretest bioassay (serial dilution assay tested for *Bacillus subtilis*, *Escherichia coli*, and *Candida albicans*). Compounds **8/9** (tested as an inseparable mixture, see above), **10**, **11**, and **13** showed weak antimicrobial activity against *Bacillus subtilis*; compounds **8/9** and **13** demonstrated weak activities against *Micrococcus luteus*; compounds **4**, **8/9**, and **13** exhibited weak activities against *Staphylococcus aureus*. Further, most of the tested compounds demonstrated moderate activity against *Mucor hiemalis*, except for compound **7**, which was inactive. No activity against Gram- bacteria or yeast was observed. In the cytotoxicity assay **4**, **8–11**, and **13** exhibited weak effects against KB 3.1 cells, while **10** and **13** showed weak activity against L929 cells.

Notably, none of the isolated metabolites has reached the initially observed MIC of the crude extracts. Therefore, we cannot exclude cumulative effects. However, it might also be possible that we have lost some potent components due to its instability during the isolation procedure or that some of the minor congeners we obtained that were not tested for lack of material could be more active.

EXPERIMENTAL SECTION

General Experimental Procedures. CD spectra were determined with a JASCO spectropolarimeter, model J-815 (JASCO, Pfungstadt, Germany). Optical rotations were recorded on a

Table 4. Antimicrobial and Cytotoxic Activities of Compounds 4–14^a

test organisms	4	5	7	8/9	10	11	12	13	14	reference
MIC ($\mu\text{g/mL}$)										
Gram-Positive Bacteria										
<i>Bacillus subtilis</i> DSM 10	—	—	—	100	75	75	—	100	—	ciprofloxacin 3.1
<i>Micrococcus luteus</i> DSM 1790	—	—	—	100	/	—	—	100	—	oxytetracycline 0.4
<i>Mycobacterium smegmatis</i> DSM 43524	—	—	—	—	/	—	—	—	—	kanamycin 0.4
<i>Staphylococcus aureus</i> DSM 346	100	—	—	100	/	—	—	100	—	oxytetracycline 0.4
Filamentous Fungi										
<i>Mucor hiemalis</i> DSM 2656	100	50	—	100	/	50	50	25	50	nystatin 16.7
Cytotoxicity IC ₅₀ (μM)										
Cell Lines										
L929	/	/	/	—	59.8	—	—	117.5	—	epothilon B 0.00275
KB3.1	172.9	/	/	144.5	27.8	141	—	79.8	—	epothilon B 6.5×10^{-5}

^a— no activity; / not tested; starting concentrations for antimicrobial assay and cytotoxicity assay were 300 and 37 $\mu\text{g/mL}$, respectively.

PerkinElmer (Überlingen, Germany) 241 MC polarimeter. NMR spectra were recorded on Bruker 700 spectrometers with a 5 mm TXI cryoprobe (^1H 700 MHz, ^{13}C 175 MHz, ^{15}N 71 MHz) and Bruker AV III-600 (^1H 600 MHz, ^{13}C 150 MHz) spectrometers. HR-ESIMS mass spectra were measured by using an Agilent 1200 series HPLC-UV system (column 2.1×50 mm, $1.7 \mu\text{m}$, C_{18} Acquity UPLC BEH (waters), solvent A (H_2O + 0.1% formic acid); solvent B (ACN + 0.1% formic acid), gradient: 5% B for 0.5 min increasing to 100% in 19.5 min and then maintaining 100% B for 5 min, flow rate 0.6 mL/min, UV/vis detection 200–600 nm combined with ESI-TOF-MS (Maxis, Bruker)) [scan range 100–2500 m/z , capillary voltage 4500 V, dry temperature 200 °C]. UV spectra were recorded by using a Shimadzu UV-2450 UV–vis spectrophotometer.

Fungal Material. The fungal specimen was collected by C. Decock and J. C. Matasyoh from Mount Elgon National Reserve, located in the western part of Kenya ($1^\circ 7' 6''$ N, $34^\circ 31' 30''$ E) in April 2016 from wood. A dried specimen and the corresponding mycelial culture, which was obtained from the contents of the basidome, were deposited at MUCL, Louvain-la-Neuve, Belgium, under the accession number MUCL 56354.

DNA was extracted from the culture of MUCL 56354 by using the EZ-10 spin column genomic DNA miniprep kit (Bio Basic Canada Inc., Markham, Ontario, Canada) as described previously.¹² A Precellys 24 homogenizer (Bertin Technologies, France) was used for cell disruption at a speed of 6000 rpm for 2×40 s. Standard primers ITS 1f and NL4 were used for the DNA region amplification by following the protocol by Otto et al.²²

Small-Scale Fermentation. *Sanghuangporous* sp. MUCL56354 was cultivated in three different liquid media: YM 6.3, Q6_{1/2}, and ZM_{1/2} (media compositions see Supporting Information). A well-grown culture from a YM agar plate (YM supplemented with 1.5% agar, pH = 6.3) was cut into small pieces using a cork borer (7 mm), and five pieces were inoculated into a 500 mL Erlenmeyer flask containing 200 mL of the three media. The cultures were incubated at 23 °C on a rotary shaker (140 rpm). The growth of the fungus was monitored by measuring the amount of free glucose using Diastix Harnzuckerstreifen (Bayer). The fermentation was terminated 3 days after glucose depletion.

Scale-up of Fermentation. Analysis of the HPLC-MS data and subsequent search in the Dictionary of Natural Products database²³ revealed the presence of potentially new bioactive metabolites in the YM 6.3 medium, and this was selected for scale-up. A well-grown YM agar plate of mycelial culture was cut into small pieces using a 7 mm cork borer and five pieces inoculated into larger batches of sterile flasks (in total 10 L) by following the original protocol.

Preparation of the Extracts. Supernatant and biomass from small-scale fermentation were separated by filtration. The supernatant resulting from an ethyl acetate extract was evaporated to dryness by means of a rotary evaporator. The mycelia were extracted with 200 mL of acetone in an ultrasonic bath for 30 min and filtered, and the filtrate was evaporated. The remaining water phase was suspended in

an equal amount of distilled water, extracted with an equal amount of ethyl acetate, and filtered through anhydrous sodium sulfate, and the resulting ethyl acetate extract was evaporated to dryness.

The mycelia and supernatant from the large-scale fermentation were separated via vacuum filtration. The mycelia were extracted with 4×500 mL of acetone in an ultrasonic bath for 30 min. The extracts were combined and the solvent was evaporated by means of a rotary evaporator. The remaining water phase was suspended in 200 mL of distilled water, extracted with 3×500 mL of ethyl acetate, and filtered through anhydrous sodium sulfate. The resulting ethyl acetate extract was evaporated to dryness, leaving a brown solid (151 mg). The supernatant was extracted by adding 5% Amberlite XAD-16N absorbent (Rohm & Haas Deutschland GmbH, Frankfurt am Main, Germany) and incubation of the resin overnight on a shaker. The Amberlite resin was then filtered and eluted with 4×500 mL of acetone. The resulting acetone extract was evaporated, and the remaining water phase was extracted with an equal amount of ethyl acetate. The organic phase was dried by sodium sulfate and evaporated to dryness, and a brown extract (369 mg) was obtained.

Isolation of Compounds 1–14. The extract was filtered using a SPME Strata-X 33 u Polymeric RP cartridge (Phenomenex, Inc., Aschaffenburg, Germany). The supernatant and mycelia crude extracts were fractionated by preparative reverse-phase liquid chromatography (PLC 2020, Gilson, Middleton, WI, USA). The VP Nucleodur 100–5C 18 ec column (250 mm $\times$ 40 mm, $7 \mu\text{m}$, Macherey-Nagel) was used as stationary phase. Deionized water (Milli-Q, Millipore, Schwalbach, Germany) with 0.05% trifluoroacetic acid (TFA) (solvent A) and acetonitrile with 0.05% TFA (solvent B) were used as the mobile phase. The elution gradient used 5–100% solvent B over 45 min and thereafter isocratic conditions at 100% solvent B for 10 min. UV monitoring was carried out at 210, 254, and 350 nm, and the flow rate was 40 mL/min. Twenty-four fractions were collected according to the observed peaks (F1–F24) from supernatant extracts, and 14 fractions (F1–F14) were collected from the mycelial extracts.

Fraction F15 of the supernatant was further purified by reversed-phase HPLC (solvent A (H_2O + 0.05% TFA)/solvent B (ACN + 0.05% TFA), elution gradient: 52–100% solvent B for 23 min, followed by maintaining isocratic conditions at 100% for 5 min with a preparative HPLC column (Kromasil, MZ Analysentechnik, Mainz, Germany; 250×20 mm, $7 \mu\text{m}$ C_{18}) as stationary phase and a flow rate of 15 mL/min, to afford compound 1 (0.42 mg; equivalent to 0.1% of the crude extract). Unless stated otherwise, the same column was used for the purification of the other fractions. Fraction F12 and supernatant fraction F23 were combined and purified with a gradient of 60–85% solvent B for 18 min, followed by a gradient shift from 85% to 100% in 2 min and isocratic conditions at 100% for 5 min to afford compounds 2 (0.28 mg; 0.05%), 3 (0.49 mg; 0.1%), 8/9 (3.31 mg; 0.65%), and 11 (2.55 mg; 0.5%). Application of the elution gradient of 40–80% solvent B for 18 min, followed by gradient shift from 80% to 100% in 2 min and maintaining isocratic conditions

100% for 5 min, yielded compound 4 (1.51 mg; 0.4%) and compound 7 (4.08 mg; 1.1%) from fraction F18 of the supernatant. Compound 5 (3.07 mg; 0.8%) was purified from supernatant fraction F17 with the following elution gradient: 60–100% solvent B for 23 min, followed by isocratic conditions at 100% B for 5 min. Compound 6 (0.89 mg; 0.25%) was purified from supernatant fraction F11 by application of the following gradient: 38–62% solvent B for 23 min, followed by gradient shift from 62% to 100% in 2 min and isocratic gradient 100% for 5 min. The same gradient was used to isolate compound 10 (1.34 mg; 0.35%) from supernatant fraction F20. Fraction F15 of the supernatant was purified using the elution gradient 38–75% solvent B for 23 min, followed by a gradient shift from 75% to 100% in 2 min and isocratic conditions of 100% solvent B for 2 min to afford compound 12 (1.2 mg; 0.3%). Compound 13 (2.17 mg; 0.4%) was obtained from purification of supernatant fraction F21 and mycelia fraction F10 with the gradient 55–85% solvent B for 18 min, followed by 2 min gradient shift from 85% to 100% and finally isocratic conditions at 100% B for 5 min. The known compound 14 (6.79 mg; 2%) was purified from supernatant fraction F7 with the elution gradient 34–100% solvent B for 23 min, followed by isocratic elution with 100% B for 5 min.

Antimicrobial Assay. Minimum inhibitory concentrations (MIC) were determined in serial dilution assays using different test microorganisms. The assays were conducted in 96-well plates in Mueller Hinton Broth (comprising beef infusion solids, 2.0 g/L; casein hydrolysate, 17.5 g/L; starch, 1.5 g/L) for bacteria and YM medium for filamentous fungi and yeasts, as described earlier.²⁴

Cytotoxicity Assay. In vitro cytotoxicity (IC₅₀) of compounds 3, 4, and 8–14 were evaluated against mouse fibroblast L929 and HeLa (KB-3.1) cell lines according to the method described earlier.⁶

(6*R*,7*S*,10*R*)-7,10-Epoxy-7,11-dimethyldodec-1-ene-6,11-diol (**1**): [α]_D²⁰ +40.7 (c 1.075, MeOH); UV (MeOH, ϵ 0.125) $\lambda_{\max}$ (log ϵ) 222 (3.828); HR-ESIMS m/z 255.1954 [M + H]⁺, calcd for C₁₅H₂₇O₃ 255.1960.

(9*R*)-9-[(8*S*)-8-Hydroxy-7-[(6*R*)-3-methylcyclohex-3-en-6-yl]prop-7-en-8-yl]-11-methylfuran-12-one (elgonene **A** (**2**)): [α]_D²⁰ +82.3 (c 1.0, MeOH); UV (MeOH, ϵ 0.125) $\lambda_{\max}$ (log ϵ) 222 (3.879); HR-ESIMS m/z 249.1486 [M + H]⁺, calcd for C₁₅H₂₁O₃ 249.1490.

(9*R*,11*R*)-9-[(8*S*)-8-Hydroxy-7-[(6*R*)-3-methylcyclohex-3-en-6-yl]prop-7-en-8-yl]-11-methyloxolan-12-one (elgonene **B** (**3**)): [α]_D²⁰ +14.3 (c 1.0, MeOH); UV (MeOH, ϵ 0.125) $\lambda_{\max}$ (log ϵ) 230 (3.659); HR-ESIMS m/z 251.1641 [M + H]⁺, calcd for C₁₅H₂₃O₃ 251.1647.

(2*E*,4*S*,5*S*)-4,5-Dihydroxy-2-methyl-6-[(7*R*)-10-methylcyclohex-10-en-7-yl]hepta-2,6-dienoic acid (elgonene **C** (**4**)): [α]_D²⁰ +25.9 (c 2.0, MeOH); UV (MeOH, ϵ 0.125) $\lambda_{\max}$ (log ϵ) 281 (2.589), 203 (3.849); HR-ESIMS m/z 267.1588 [M + H]⁺, calcd for C₁₅H₂₃O₃ 267.1596.

(2*E*,5*S*,6*R*)-5,6-Dihydroxy-2-methyl-6-[(7*R*)-10-methylcyclohex-10-en-7-yl]hept-2-enoic acid (elgonene **D** (**5**)): [α]_D²⁰ −12.2 (c 1.99, MeOH); UV (MeOH, ϵ 0.1) $\lambda_{\max}$ (log ϵ) 197 (3.986); HR-ESIMS m/z 269.1743 [M + H]⁺, calcd for C₁₅H₂₅O₄ 269.1752.

(2*E*,5*S*,6*R*)-5,6-Dihydroxy-2-methyl-6-[(7*R*)-10-(hydroxymethyl)cyclohex-10-en-7-yl]-2-methylhept-2-enoic acid (elgonene **E** (**6**)): [α]_D²⁰ +61.1 (c 1.49, MeOH); UV (MeOH, ϵ 0.0) $\lambda_{\max}$ (log ϵ) 218 (3.890); HR-ESIMS m/z 285.1694 [M + H]⁺, calcd for C₁₅H₂₅O₅ 285.1702.

(2*E*,4*S*,5*S*,6*R*)-4,5,6-Trihydroxy-2-methyl-6-[(7*R*)-10-methylcyclohex-10-en-7-yl]hept-2-enoic acid (elgonene **F** (**7**)): [α]_D²⁰ −20.5 (c 1.29, MeOH); UV (MeOH, ϵ 0.05) $\lambda_{\max}$ (log ϵ) 212 (4.103); HR-ESIMS m/z 285.1695 [M + H]⁺, calcd for C₁₅H₂₅O₅ 285.1702.

(9*R*)-11-Methyl-9-[(7*R*,8*R*)-7-methyl-6-[(6*R*)-3-methylcyclohex-3-en-6-yl]oxiran-8-yl]furan-12-one (elgonene **G** (**8**)): UV (MeOH, ϵ 0.125) $\lambda_{\max}$ (log ϵ) 216 (3.879); HR-ESIMS m/z 249.1486 [M + H]⁺, calcd for C₁₅H₂₁O₃ 249.1490.

(9*R*,11*R*)-11-Methyl-9-[(7*R*,8*R*)-7-methyl-6-[(6*R*)-3-methylcyclohex-3-en-6-yl]oxiran-8-yl]oxolan-12-one (elgonene **H** (**9**)): UV (MeOH, ϵ 0.125) $\lambda_{\max}$ (log ϵ) 216 (3.879); HR-ESIMS m/z 251.1639 [M + H]⁺, calcd for C₁₅H₂₃O₃ 251.1647.

{(5*R*,6*R*)-5-[9-(Furan-9-yl)ethyl]-5-methyl-1-methylidenecyclohexyl}methanol (elgonene **I** (**10**)): [α]_D²⁰ −11.9 (c

2.0, MeOH); UV (MeOH, ϵ 0.125) $\lambda_{\max}$ (log ϵ) 228 (3.8453); HR-ESIMS m/z 235.1689 [M + H]⁺, calcd for C₁₅H₂₃O₂ 235.1698.

(5*R*,6*R*)-5-[9-(Furan-9-yl)ethyl]-5-methyl-1-methylidenecyclohexane-5-carboxylic acid (elgonene **J** (**11**)): [α]_D²⁰ +11.9 (c 3.6, MeOH); UV (MeOH, ϵ 0.25) $\lambda_{\max}$ (log ϵ) 277(2.894), 216 (3.834); HR-ESIMS m/z 249.1483 [M + H]⁺, calcd for C₁₅H₂₁O₃ 249.1490.

(9*Z*)-9-[9-[(5*R*,6*R*)-5-(Hydroxymethyl)-5-methyl-1-methylidenecyclohexyl]ethyl]but-9-ene-11,15-diol (elgonene **K** (**12**)): [α]_D²⁰ +10.8 (c 10.41, MeOH); UV (MeOH, ϵ 0.125) $\lambda_{\max}$ (log ϵ) 203 (3.849); HR-ESIMS m/z 255.1954 [M + H]⁺, calcd for C₁₅H₂₇O₃ 255.1960.

(9*Z*)-9-[9-[(6*S*)-5,5-Dimethyl-1-methylidenecyclohexyl]ethyl]but-9-ene-11,15-diol (elgonene **L** (**13**)): [α]_D²⁰ +7.9 (c 1.24, MeOH); UV (MeOH, ϵ 0.25) $\lambda_{\max}$ (log ϵ) 213 (3.634); HR-ESIMS m/z 239.2002 [M + H]⁺, calcd for C₁₅H₂₇O₂ 239.2011.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.8b01086.

Experimental procedures, 1D and 2D NMR data, LCMS data, 5.8S/ITS DNA sequence of the producing organism (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Tetersoo, L.; Bahram, M.; Pölme, S.; Köljal, U.; Yorou, N. S.; Wijesundera, R.; Villarreal Ruiz, L.; Vasco-Palacios, A. M.; Thu, P. Q.; Suija, A.; Smith, M. E.; Sharp, C.; Saluveer, E.; Saitta, A.; Rosas, M.; Riit, T.; Ratkowsky, D.; Pritsch, K.; Pöldmaa, K.; Piepenbring, M.; Phosri, C.; Peterson, M.; Parts, K.; Pärtel, K.; Otsing, E.; Nouhra, E.; Njouonkou, A. L.; Nilsson, R. H.; Morgado, L. N.; Mayor, J.; May, T. W.; Majuakim, L.; Lodge, D. J.; Lee, S. S.; Larsson, K. H.; Kohout, P.; Hosaka, K.; Hiiesalu, I.; Henkel, T. W.; Harend, H.; Guo, L. D.; Greslebin, A.; Grelet, G.; Geml, J.; Gates, G.; Dunstan, W.; Dunk, C.; Drenkhan, R.; Dearnaley, J.; De Kesel, A.; Dang, T.; Chen, X.; Buegger, F.; Brearley, F. Q.; Bonito, G.; Anslan, S.; Abell, S.; Abarenkov, K. *Science* **2014**, *346*, 1052–1053.
- (2) Sandargo, B.; Chepkirui, C.; Cheng, T.; Chaverra-Munoz, L.; Thongbai, B.; Stadler, M.; Hüttel, S. *Biotechnol. Adv.* **2019**, DOI: 10.1016/j.biotechadv.2019.01.011.
- (3) Chepkirui, C.; Richter, C.; Matasyoh, J. C.; Stadler, M. *Phytochemistry* **2016**, *132*, 95–101.

- (4) Chepkirui, C.; Matasyoh, J. C.; Decock, C.; Stadler, M. *Phytochem. Lett.* **2017**, *20*, 106–110.
- (5) Chepkirui, C.; Cheng, T.; Matasyoh, J. C.; Decock, C.; Stadler, M. *Phytochem. Lett.* **2018**, *25*, 141–146.
- (6) Chepkirui, C.; Sum, C. W.; Cheng, T.; Matasyoh, J. C.; Decock, C.; Stadler, M. *Molecules* **2018**, *23*, 369.
- (7) Chepkirui, C.; Yuyama, K. T.; Wanga, L. A.; Decock, C.; Matasyoh, J. C.; Abraham, W. R.; Stadler, M. *J. Nat. Prod.* **2018**, *81*, 778–784.
- (8) Mudalungu, C. M.; Richter, C.; Wittstein, K.; Abdalla, M. A.; Matasyoh, J. C.; Stadler, M.; Süßmuth, R. D. *J. Nat. Prod.* **2016**, *79*, 894–898.
- (9) Hsieh, P. W.; Wu, J. B.; Wu, Y. C. *BioMedicine* **2013**, *3*, 106–113.
- (10) Zhou, L. W.; Vlasák, J.; Decock, C.; Assefa, A.; Stenlid, J.; Abate, D.; Wu, S. H.; Dai, Y. C. *Fungal. Divers.* **2016**, *77*, 335–347.
- (11) Chen, H.; Tian, T.; Miao, H.; Zhao, Y. Y. *Fitoterapia* **2016**, *113*, 6–26.
- (12) Wendt, L.; Sir, E. B.; Kuhnert, E.; Heitkämper, S.; Lambert, C.; Hladki, A. I.; Romero, A. I.; Luangsa-ard, J. J.; Srikitikulchai, P.; Peršoh, D.; Stadler, M. *Mycol. Prog.* **2018**, *17*, 115–154.
- (13) Inamatsu, K. *Soil Sci. Plant Nutr.* **1968**, *14*, 62–67.
- (14) Inui, T.; Wang, Y.; Nikolic, D.; Smith, D. C.; Franzblau, S. G.; Pauli, G. F. *J. Nat. Prod.* **2010**, *73*, 563–567.
- (15) Li, L.; Liu, R.; Han, L.; Jiang, Y.; Liu, J.; Li, Y.; Yuan, C.; Huang, X. *Magn. Reson. Chem.* **2016**, *54*, 606–609.
- (16) Hoye, T. R.; Jeffrey, C. S.; Shao, F. *Nat. Protoc.* **2007**, *2*, 2451.
- (17) Yang, J.; Wang, N.; Yuan, H. S.; Hu, J. C.; Dai, Y. C. *Chem. Nat. Compd.* **2013**, *49*, 261–263.
- (18) Kobayashi, Y.; Ino, C.; Hirotani, M. Japanese Patent JP2010047512, 2010.
- (19) Fontana, A.; Muniain, C.; Cimino, G. *J. Nat. Prod.* **1998**, *61*, 1027–1029.
- (20) Thompson, J. E.; Walker, R. P.; Wratten, S. J.; Faulkner, D. J. *Tetrahedron* **1982**, *38*, 1865–1873.
- (21) Ludwiczuk, A.; Odrzykoski, I. J.; Asakawa, Y. *Phytochemistry* **2013**, *95*, 234–241.
- (22) Otto, A.; Laub, A.; Wendt, L.; Porzel, A.; Schmidt, J.; Palfner, G.; Becerra, J.; Krüger, D.; Stadler, M.; Wessjohann, L.; Westermann, B.; Arnold, N. *J. Nat. Prod.* **2016**, *79*, 929–938.
- (23) Buckingham, J. *Chapman & Hall, Chemical Database*; CRC: Boca Raton, FL, USA, 2017.
- (24) Rupcic, Z.; Chepkirui, C.; Hernández-Restrepo, M.; Crous, P.; Luangsa-ard, J. J.; Stadler, M. *MycKeys* **2018**, *33*, 1–23.