Caryophyllenes from a Fungal Culture of *Chrysosporium pilosum*[⊥]

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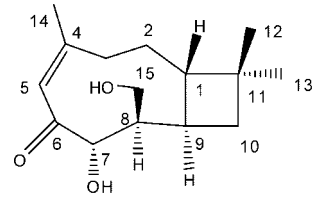
Four new caryophyllene derivatives, Sch 725432 (**1**), Sch 601253 (**2**), Sch 601254 (**3**), and Sch 725434 (**4**), were isolated from the fungal fermentation broth of *Chrysosporium pilosum* by reversed-phase HPLC purification. The structure elucidation of trioxygenated caryophyllenes **1**−**4** was accomplished on the basis of spectroscopic data interpretation. Sch 725434 (**4**) possesses a dihydrofuran-3-one ring, forming a tricyclic ring skeleton, which represents an unprecedented ring skeleton for the caryophyllene-type of sesquiterpenes. Compounds **1**−**4** were evaluated for their antifungal activity.

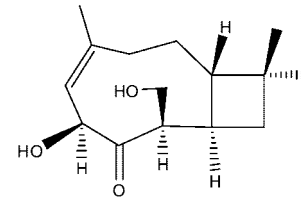
Caryophyllene and its related analogues have been widely isolated from plants, such as black pepper (*Piper nigrum* L.),[1] cinnamon (*Cinnamomum* spp.),[2] oregano (*Origanum vulgare* L.),[3] clary sage oil (*Salvia sclarea* L.),[4] *Lychnophora affinis* Gardn.,[5] *Inula spiraefolia*,[6] and *Pulicaria* sp.[7−9] Caryophyllenes have also been discovered from various fungal species, such as *Pestalotiopsis* sp.,[10] *Wallemia sebi*,[11] *Poronia punctata*,[12,13] *Naematoloma fasciculare*,[14] and *Hypoxylon terricola*.[15] Due to their advantageous taste and odor, some caryophyllene analogues have been used as flavors, food additives, and fragrances and in perfumery and cosmetics.[16−18] Certain caryophyllene derivatives have been reported to display antimicrobial activity[19] and cytotoxic activity or immunosuppressive activity.[10] Most recently, (*E*)-*β*-caryophyllene was identified as a potent and selective cannabinoid receptor type 2 (CB2) agonist.[20] It exhibited a strong oral anti-inflammatory effect in a wild-type mouse model but not in a CB2 knockout mouse model.[20]

In the course of our drug discovery program searching for antimicrobial lead candidates, a large number of extracts from natural sources have been evaluated in an antimicrobial assay. The screening of these extracts derived from fungi and bacteria from terrestrial and marine organisms has led to the discovery of various antimicrobial compounds.[21−24] Previously, we identified an antifungal sterol sulfate, Sch 601324, produced by a fungal culture designated as *Chrysosporium pilosum* (family Onygenaceae).[21] Further investigation of secondary metabolites from this microorganism led to the discovery of four novel caryophyllene sesquiterpenes (**1**−**4**). Described herein are the fermentation, isolation, and structure elucidation of **1**−**4**.

The microorganism SPRI-IL15503 from our microbial collection was purchased from a commercial source. Taxonomy of this microorganism was determined by DNA sequencing and sequence similarity search from the GenBank database. The frozen stock broth of the fungal culture, *C. pilosum*, was incubated twice to obtain a seed culture. Further inoculation of the seed culture to the fermentation medium and subsequent incubation afforded a fermentation broth. The fermentation broth was extracted with acetonitrile (MeCN). The MeCN extract was further fractionated with a solid-phase extraction method. Pure compounds **1**−**4** were obtained after reversed-phase HPLC purification.



Sch 725432 (**1**)



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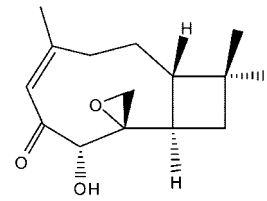

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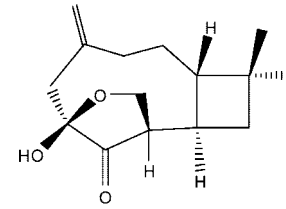
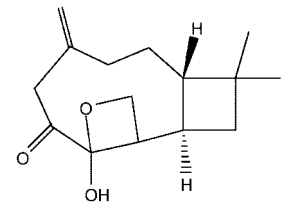


Sch 725434 (**4**)



4a

From a high-resolution ESIMS measurement, the molecular formula of Sch 725432 (**1**) was established as $C_{15}H_{24}O_3$. The structure of **1** was further elucidated by extensive NMR data analysis (Tables 1 and 2). Fifteen carbons were observed in the ^{13}C NMR spectrum, as shown in Table 2, suggesting a sesquiterpene skeleton that contained one secondary and one tertiary hydroxyl group (δ 58.2, 74.2, respectively) and an α,β-unsaturated ketone (δ 126.5, 148.9, and 207.5). All proton signals in the ^1H NMR spectrum (Table 1) were interpreted and assigned to their adjacent carbons by analysis of HSQC data. Thus, three methyl groups (δ 0.87, 0.91, and 1.81, all singlets), a vinyl proton (δ 6.11), a proton of an oxygen-bearing methine (δ 4.31), and two protons of an oxygen-bearing methylene (δ 3.64, 3.71) were characterized. Two hydroxyl protons (δ 4.52 and 5.05) were identified due to the lack of corresponding cross-peaks observed in the HSQC spectrum. The sesquiterpene skeleton was constructed further by interpretation of the HSQC-TOCSY and HMBC data (Table S1, Supporting Information). Connectivity of the proton-attached carbon framework was assembled by analysis of the HSQC-TOCSY spectrum.

[⊥] Dedicated to Dr. David G. I. Kingston of Virginia Polytechnic Institute and State University for his pioneering work on bioactive natural products.

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Table 1. ^{1}H NMR Spectroscopic Data of Compounds **1**−**4**[a]

	1	**2**	**3**	**4**
position	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
1	1.60, m	1.51, m	1.93, ddd (10.7, 9, 3.7)	2.01, ddd (12.0, 9.0,4.0)
2α	1.20, br t (12)	1.11, m	1.59, dddd (14.6, 10.7, 10.7, 3.7)	1.28, m
2β	1.47, m	1.43, m	1.74, dddd (14.6, 10.7, 6.8, 3.9)	1.43, m
3	1.99, dd (13.6, 8.6)	1.63, m	2.48, m	1.45, m
	2.31, m	2.02, td (13.7, 5.4)	2.55, m	2.03, m
5	6.11, s	5.66, d (9.1)	6.10, s	2.38, s
6		4.15, dd (9.1, 4.3)		
7	4.31, t (4.4)		4.52, s	
8	1.56, m	2.65, ddd (10.7, 10.7, 4.1)		2.33, q (3.3)
9	2.29, m	1.72, m	2.57, q (9)	2.13, qd (9.2, 3.0)
10	1.53, m	1.28, dd (10.3, 9.7)	1.34, m	1.39, dd (11, 10)
		1.61, dd (10.3, 7.8)		1.65, dd (11, 8.8)
12	0.91, s	0.94, s	0.95, s	0.97, s
13	0.87, s	0.88, s	0.98, s	0.92, s
14	1.81, s	1.65, s	1.98, d (1.2)	4.80, t (1.8) 4.89, brs
15	3.64, dd (11, 7.8)[b]	3.28, m	2.42, d (5)	4.15, d (3.3)
	3.71, d (11, 4.4)[b]	3.37, m	2.81, d (5)	
OH-6		4.95, d (4.3)		6.53, s
OH-7	5.05, d (4.4)			
OH-15	4.52, br s	4.50, s		

[a] The NMR data of compounds **1**, **2**, and **4** were acquired in DMSO-d_6, whereas the data of compound **3** were acquired in CD_3OD. [b] J values were calculated from a ^{1}H NMR spectrum acquired in CD_3OD.

Table 2. ^{13}C NMR Spectroscopic Data of Compounds **1**−**4**[a]

	1	**2**	**3**	**4**
position	δ_C, mult.[b]	δ_C, mult.[b]	δ_C, mult.[b]	δ_C, mult.[b]
1	44.9, CH	48.8, CH	48.6, CH	42.0, CH
2	25.9, CH_2	23.4, CH_2	26.3, CH_2	27.7, CH_2
3	30.4, CH_2	27.7, CH_2	33.8, CH_2	27.8, CH_2
4	148.9, qC	142.7, qC	158.0, qC	142.8, qC
5	126.5, CH	120.8, CH	127.3, CH	46.0, CH_2
6	207.5, qC	76.4, CH	203.0, qC	97.7, qC
7	74.2, CH	215.2, qC	77.6, CH	216.6, qC
8	50.2, CH	57.7, CH	62.4, qC	48.3, CH
9	31.2, CH	35.8, CH	35.0, CH	36.7, CH
10	37.4, CH_2	37.9, CH_2	33.5, CH_2	37.2, CH_2
11	32.9, qC	34.1, qC	34.5, qC	33.4, qC
12	29.8, CH_3	29.8, CH_3	30.2, CH_3	29.4, CH_3
13	23.3, CH_3	22.1, CH_3	23.9, CH_3	23.1, CH_3
14	25.2, CH_3	21.8, CH_3	28.1, CH_3	115.1, CH_2
15	58.2, CH_2	62.0, CH_2	48.0, CH_2	63.5, CH_2

[a] The NMR data of compounds **1**, **2**, and **4** were acquired in DMSO-d_6, whereas the data of compound **3** were acquired in CD_3OD. [b] One-bond ^{1}H−^{13}C correlations were observed in the HSQC spectrum. Two-bond ^{1}H−^{13}C correlations were observed in HSQC-TOCSY when the neighboring carbons were proton-bearing carbons.

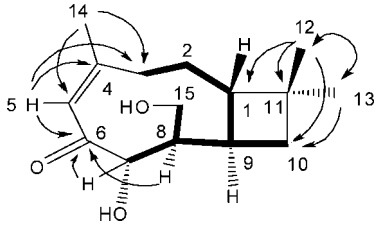

Figure 1. Key 2D NMR correlations of **1**. Bolded lines: connectivity determined by HSQC-TOCSY analysis. Arrow: Key ^{1}H−^{13}C long-range correlations observed in the HMBC spectrum.

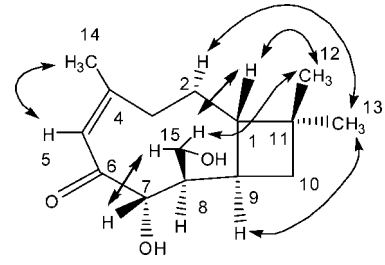

Figure 2. Key NOE correlations of **1**.

Corresponding couplings established the following connectivities: C-1 through C-3; C-7 through C-10; C-1 and C-9; C-8 and C-15, as shown in Figure 1. The location of C-11 (δ 32.9) was assigned to the quaternary center of C-1, C-10, and two methyl groups (C-12 and C-13), on the basis of the long-range correlations of H_3-12 and H_3-13 to C-1, C-10, and C-11, as observed in the HMBC spectrum. This connectivity formed a *gem*-dimethyl-substituted cyclobutyl ring moiety. The long-range correlation of H-7 (δ 4.31) to the ketone functionality (C-6) was used to establish the attachment of carbonyl C-6 to C-7. Finally, correlations of H_3-14 to C-3, C-4, and C-5 and of H-5 to C-4 and C-6 clearly determined the vinyl methyl and alkenyl group positions. Thus, the $\Delta^{4,5}$ double bond was conjugated with the C-6 carbonyl group. This linkage was used to construct a nine-membered macrocyclic ring system. The bicyclic skeleton of **1**, therefore, belongs to the caryophyllene-type of sesquiterpenes.

The relative configuration of **1** was determined by analysis of the ^{1}H−^{1}H NMR coupling constants and NOE data. Observation of the NOE correlations of H-1, H_3-12, and H_2-15 indicated the β-orientation of these protons as shown in Figure 2. In contrast, observation of the NOE correlations of H-9 and H_3-13 revealed

the α-orientation of these protons. Thus, the *trans* configuration at the ring junction was established between the cyclobutyl and the nine-membered ring. Finally, the C-7 hydroxyl group was determined to be at an α-position on the basis of the NOE correlation between H-7 and H_2-15, to complete the structure elucidation of **1**.

Sch 601253 (**2**) was found to possess the same protonated molecular ion (*m*/*z* 253 $[M + H]^+$) in the EIMS as **1**, suggesting the same molecular formula of $C_{15}H_{24}O_3$. In the ^{13}C NMR spectrum, 15 carbon signals were observed, and they displayed similar chemical shifts and multiplicities for C-1 through C-3, and C-9 through C-15, in comparison with those of **1**. Therefore, the major structural variation between **1** and **2** should be between C-4 and C-7. Using the same analytical method, the connectivity of the proton-attached carbon scaffold, C-1 through C-3, C-8 through C-10, C-1 to C-9, and C-8 to C-15, was assigned unambiguously from the HSQC-TOCSY data. The locations of the three methyl groups (δ 21.8, 22.1, and 29.8) and one double bond (δ 120.8 and 142.7) were determined to be the same as in **1** on the basis of the following long-range HMBC correlations: H_3-12 and H_3-13 to C-1, C-10, and C-11; H-14 to C-3, C-4, and C-5 (Table S1, Supporting Information). Observation of H-6 of the oxygen-bearing methine coupled with the vinyl proton H-5 (J = 9.1 Hz) ascertained the connectivity between C-5 and C-6. The carbonyl C-7 with a downfield chemical shift δ 215.2 indicated a typical carbonyl functionality without conjugation. Furthermore, the C-7 carbonyl group was assigned to a position between C-6 and C-8 according to the long-range correlations of H-5 to C-6 and C-7 and of H_2-15 to C-7. The relative configuration of the secondary alcohol at C-15 was determined as β, due to the observation of a NOE correlation between H_2-15 and H-1. The NOE correlation between H-6 and H-8 was used to establish the stereochemistry of C-6 (Table S2, Supporting Information). Compound **2** was proposed as a regioisomer of **1**, with the two functional groups switched at C-6 and C-7.

The molecular formula of Sch 601254 (**3**) was established as $C_{15}H_{22}O_3$, two protons less than that in **1** on the basis of the HREIMS data. On comparing the ^1H and ^{13}C NMR spectra of **1** and **3**, compound **3** was revealed to be a closely related analogue of **1**. The major distinction from the two ^{13}C NMR spectra was that the tertiary carbon C-8 (δ 50.2) of **1** transformed to an oxygen-bearing quaternary carbon (δ 62.4 s) in **3**. The ^1H and ^{13}C NMR data of the oxygenated methylene (C-15, δ 48.0; H_2-15, δ 2.42, d, J = 5 Hz, and δ 2.81, d, J = 5 Hz) and oxygenated quaternary carbon (C-8) of **3** demonstrated distinct characteristics for the presence of an epoxide functionality. Therefore, compound **3** was proposed as the 8,15-epoxide derivative of **1**. Two-dimensional NMR studies were performed to confirm this proposed structure. The proton-attached carbon framework, C-1 through C-3, C-9 to C-10, and C-1 to C-9, was established by analysis of the HSQC-TOCSY data. The locations of three methyl groups (C-13, δ 23.9; C-14, δ 28.1; C-12, δ 30.2), a double bond (C-5, δ 127.3; C-4, δ 158.0), and one carbonyl group (C-6, δ 203) were verified to be the same as those of **1** on the basis of the corresponding long-range HMBC correlations. The attachment of the tertiary alcohol C-7 (δ 77.6) to carbonyl C-6 and quaternary C-8 was confirmed by the following long-range correlations: H-7 to C-8 and C-15; H-5 to C-7; and H_2-15 to C-7. Thus, the structure of **3** was elucidated unambiguously as the 8,15-epoxide analogue of **1**. The relative configuration of **3** was determined as being identical to that of **1** due to similar patterns of NOE correlations. The stereochemistry of C-7 and C-8 in **3** was established on the basis of NOE correlations of H_2-15 to H-7 and H-10 and the lack of any NOE correlation between H_2-15 and H-9.

Compound **4** was also identified as a sesquiterpene with a molecular formula of $C_{15}H_{22}O_3$, based on its HREIMS data. The proton-attached carbon scaffold, C-1 through C-3, C-8 through C-10, C-1 to C-9, and C-8 to C-15, was determined by analysis of the HSQC-TOCSY data, as shown in Figure 3. The two methyl groups were determined as having *gem*-dimethyl (C-12 and C-13) substitution on a cyclobutyl ring, according to the long-range $^1H-^{13}C$ couplings of H_3-12 and H_3-13 to C-1, C-10, and C-11 (Table S1, Supporting Information). A terminal olefin group ($\Delta^{4,14}$) was assigned between two methylene carbons at C-3 and C-5 on the basis of the observation of long-range correlations of H_2-14 to C-3 (δ 27.8) and C-5 (δ 46.0) and of H_2-5 to C-3, C-4, and C-14, as shown in Figure 3. Only two carbons remained unassigned: a carbonyl carbon (δ 216.6) and a quaternary acetal or hemiacetal carbon (δ 97.7). This information led to two possible structures, **4** or **4a**, for further determination. The oxygen atom on C-15 could form a hemiacetal functionality with either C-6 or C-7. The hydroxyl proton (O*H*-6) displayed correlations with the methylene (C-5), hemiacetal (C-6), and carbonyl (C-7) carbons in the HMBC spectrum, which confirmed the acetal group location between C-5 and carbonyl C-7. The oxygenated-methylene protons (H_2-15) showed a three-bond correlation with carbonyl carbon C-7. Thus, structure **4a** was ruled out since the above-described correlations were inconsistent with this proposed **4a**. Therefore the two-dimensional structure of **4** was established. The *trans* configuration of the ring junction of the cyclobutyl and macrocyclic rings was determined using a similar analysis to that described for **1**. The relative stereochemistry of C-15 was assigned as β, due to the observed NOE correlation between H-1 and H_2-15 and the lack of any NOE correlation between H-9 and H_2-15. The small coupling constant observed between H-8 and H-9 (J = 3 Hz) was also consistent with the structure. Therefore, the stereochemistry of the fused dihydrofuran-3-one ring was assigned as shown in Figure 3, and the structure elucidation of **4** was completed. To the best of our knowledge, the carbon skeleton of **4** has not been previously disclosed in the literature. The 2-hydroxydihydrofuran-3(2*H*)-one moiety alone rarely occurs among natural products.[25,26]

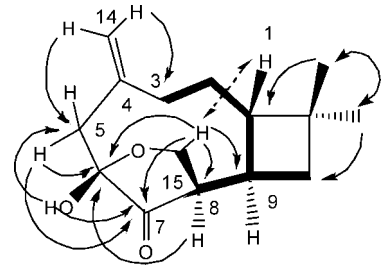

Figure 3. Key 2D NMR correlations of **4**. Bolded lines: connectivity determined by HSQC-TOCSY analysis. Arrows: Key $^1H-^{13}C$ long-range correlations observed in the HMBC spectrum. Dashed arrow: Key NOE correlation.

All of the four identified compounds possess three oxygen atoms on C-6, C-7, and C-15 with different oxidation state or cyclization position. Compounds **1**−**4** did not show any inhibition zones in a *Saccharomyces cerevisiae* (PM503) agar assay during the bioassay-guided fractionation of Sch 601324.[21] However, compound **1** showed some antifungal activity, with an MIC value of 60 μg/mL against *S. cerevisiae* (PM503). Compounds **2**−**4** were not further tested for other antimicrobial activities due to limited amount of material available.

Experimental Section

General Experimental Procedures. The NMR spectra were acquired on a Varian Unity 500 NMR instrument at 500 MHz for ^1H and 125 MHz for ^{13}C, using standard Varian pulse sequence programs (VNMR Version 6.1 software). High-resolution MS was measured on a PE Sciex QSTAR mass spectrometer.

Microorganism. The microorganism from our microbial collection was purchased from a commercial source and was designated as SPRI-IL15503. Taxonomy of this microorganism was determined by DNA sequencing since sporulating culture could not be obtained for morphol-

ogy analysis. Its genomic DNA was prepared, amplified, and sequenced. Three regions of the fungal ribosomal operon were amplified to determine species level, family and order level, and genus and family level, respectively, based on the following three regions: the Internal Transcribed Spacer regions (ITS1 & ITS2) flanking the conserved 5.8S rDNA; the first 500 bp of the 18S rDNA; and the D2 region of the 28S rDNA. From a GenBank database similarity search, the SPRI-IL15503 query for the species-specific ITS region gave an E value of e-127 for *Chrysosporium pilosum*, a member of the Onygenales. This is considered a very low E value and thus a very strong match. The bit score was 460 with an identity value of ~98%. Thus, SPRI-IL15503 isolate was assigned to *C. pilosum*.[27] The 18S and D2 28S rDNA data supported the similar relationship, albeit at higher taxonomic levels, within the Onygenales.

Fermentation. Fermentation procedure was described previously.[21]

Extraction and Isolation. The fermentation culture broth (~8 L) was stirred with 400 g of NaCl and 16 L of MeCN. The organic layer was separated and evaporated under vacuum. The extract was absorbed onto the polymeric resin, CG161 (~100 mL), and the NaCl salt was washed out with water (200 mL). The selected absorbed organic material was removed from the resin with 150 mL of 80% MeCN. After removal of solvent under vacuum, 983 mg of organic material was obtained. The crude material was fractionated on an HPLC semipreparative ODS-A column (YMC, 120 Å, S-7, 2 cm × 25 cm). The column was eluted with a gradient of MeCN−H_2O: 1−40% MeCN in 50 min, then 40% isocratic for 20 min, followed by 40−100% MeCN in another 50 min, with a flow rate of 15 mL/min. Fractions were collected (13 mL/fraction) by a fraction collector with a 150 mg sample injection run. After multiple injections and collections, pure **1** (5 mg), **2** (2 mg), **3** (1.5 mg), and **4** (2 mg) were obtained from fractions 43, 45, 55, and 72, respectively.

Sch 725432 (1): colorless gum; NMR data, see Tables 1 and 2; HREIMS *m/z* 253.1802 (calcd for $C_{15}H_{24}O_3$, 253.1798 $[M + H]^+$).

Sch 601253 (2): colorless gum; NMR data, see Tables 1 and 2; EIMS *m/z* 253 $[M + H]^+$ and 275 $[M + Na]^+$; HREIMS *m/z* 275.1618 (calcd for $C_{15}H_{24}O_3$, 275.1617 $[M + Na]^+$).

Sch 601254 (3): colorless gum; NMR data, see Tables 1 and 2; HREIMS *m/z* 273.1462 (calcd for $C_{15}H_{22}O_3$, 273.1461 $[M + Na]^+$).

Sch 725434 (4): colorless gum; NMR data, see Tables 1 and 2; HREIMS *m/z* 273.1462 (calcd for $C_{15}H_{22}O_3$, 273.1461 $[M + Na]^+$).

Antifungal Assay. In each well of a 96 well-plate, 100 μL of medium was added using a pipet. The tested compound dissolved in DMSO was 2-fold serially diluted into each well. The plates were then inoculated with 100 μL of each yeast inoculum preparation. The final concentrations of the tested compounds ranged from 64 to 1 μg/mL. The final concentration of the yeast was ~$(0.5-2.5) \times 10^3$ cfu/mL. The plate was sealed in a plastic bag and incubated at 35 °C for ~48 h. MIC values were determined after 48 h incubation by comparing the growth to the control wells using a reading mirror.

Acknowledgment. The authors are grateful to Mr. E. Langsdorf for culture fermentation and Mr. L. B. Fan for culture extraction.

Supporting Information Available: Tables of HMBC and NOE data for compounds **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Orav, A; Stulova, I.; Kailas, T.; Müürisepp, M. *J. Agric. Food Chem.* **2004**, *52*, 2582–2586.
(2) Jayaprakasha, G. K.; Jagan Mohan Rao, L.; Sakariah, K. K. *J. Agric. Food Chem.* **2003**, *51*, 4344–4348.
(3) Mockute, D.; Bernotiene, G.; Judzentiene, A. *Phytochemistry* **2001**, *57*, 65–69.
(4) Maurer, B.; Hauser, A. *Helv. Chim. Acta* **1983**, *66*, 2223–2235.
(5) Raffauf, R. F.; Pastore, M. P.; Kelley, C. J.; Le Quesne, P. W.; Miura, I; Nakanishi, K.; Finer, J.; Clardy, J. *J. Am. Chem. Soc.* **1978**, *100*, 7437–7439.
(6) Jeremic, D.; Milosavljevic, S.; Vajs, V. *Tetrahedron Lett.* **1982**, *23*, 1009–1012.
(7) Bohlmann, F.; Zdero, C. *Phytochemistry* **1981**, *20*, 2529–2534.
(8) Bohlmann, F.; Ahmed, M.; Jakupovic, J. *Phytochemistry* **1982**, *21*, 1659–1661.
(9) Hafez, S.; Sarg, T. M.; El-Domiaty; Ahmed, M.; Melek, F. R.; Bohlmann, F. *Phytochemistry* **1987**, *26*, 3356–3358.
(10) Pulici, M.; Sugawara, F.; Koshino, H.; Uzawa, J.; Yoshida, S.; Lobkovsky, E.; Clardy, J. *J. Org. Chem.* **1996**, *61*, 2122–2124.
(11) Frank, M.; Kingston, E.; Jeffery, J. C.; Moss, M. O.; Murray, M.; Simpson, T. J.; Sutherland, A. *Tetrahedron Lett.* **1999**, *40*, 133–136.
(12) Anderson, J. R.; Edwards, R. L.; Poyser, J. P.; Whalley, A. J. S. *J. Chem. Soc., Perkin Trans. I* **1988**, *823*, 831.
(13) Edwards, R. L.; Maitland, D. J.; Poyser, J. P.; Whalley, A. J. S. *J. Chem. Soc., Perkin Trans. I* **1989**, 1939–1941.
(14) Doi, K.; Shibata, T.; Yokoyama, N.; Terasawa, H.; Matsuda, O.; Kashino, S. *J. Chem. Soc., Chem. Commun.* **1990**, 725–726.
(15) Edwards, R. L.; Maitland, D. J.; Whalley, A. J. S. *J. Chem. Soc., Perkin Trans. 1* **1989**, 57–65.
(16) Maurer, B.; Hauser, A. U.S. Patent 4,522,748, 1985.
(17) Arctander, S. *Perfume and Flavor Materials of Natural Origin*; Allured Publishing Corp.: Elizabeth, NJ, 1960; p 569.
(18) Mazza, G. *Sci. Aliments* **1988**, *8*, 511–522.
(19) Poyser, J. P.; Edwards, R. L.; Hursthouse, M. B.; Walker, N. P. C.; Sheldrick, G. M.; Whalley, A. J. S. *J. Antibiot.* **1986**, *39*, 167–169.
(20) Gertsch, J.; Leonti, M.; Raduner, S.; Racz, I.; Chen, J.-Z.; Xie, X.-Q.; Altman, K.-H. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 9099–9104.
(21) Yang, S.-W.; Buevich, A.; Chan, T.-M.; Terracciano, J.; Chen, G.; Loebenberg, D.; Patel, M.; Boehm, E.; Gullo, V.; Pramanik, B.; Chu, M. *J. Antibiot.* **2003**, *56*, 419–422.
(22) Yang, S.-W.; Chan, T.-M.; Terracciano, J.; Patel, R.; Patel, M.; Gullo, V.; Chu, M. *J. Antibiot.* **2006**, *59*, 720–723.
(23) Yang, S.-W.; Chan, T.-M.; Terracciano, J.; Loebenberg, D.; Patel, M.; Gullo, V.; Chu, M. *J. Antibiot.* **2006**, *59*, 190–192.
(24) Yang, S.-W.; Chan, T. M.; Terracciano, J.; Loebenberg, D.; Patel, M.; Chu, M. *J. Antibiot.* **2005**, *58*, 535–538.
(25) Taniguchi, E.; Oshima, Y. *Agric. Biol. Chem.* **1972**, *36*, 1489–1496.
(26) Taniguchi, E.; Oshima, Y. *Agric. Biol. Chem.* **1972**, *36*, 1013–1025.
(27) Vidal, P.; Vinuesa, M. A.; Sánchez-Puelles, J M.; Guarro, J. In *Biology of Dermatophytes and Other Keratinophilic Fungi*; Kushwaha, R. K. S., Guarro, J., Eds.; Revista Iberoamericana de Micología: Bilbao, 2000; Chapter 4, pp 22−29.

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