Polyoxygenated Methyl Cyclohexanoids from a Terrestrial *Ampelomyces* Fungus

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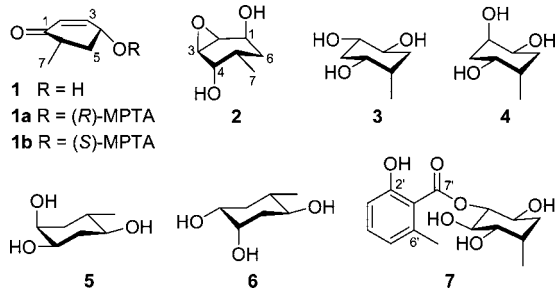
Seven structurally related new polyoxygenated methyl cyclohexanoids, ampelomins A−G (**1**−**7**), were isolated from the mycelial solid culture of a soil-derived *Ampelomyces* fungus. Their structures were determined by spectroscopic and chemical means. Ampelomins A (**1**), C (**3**), E (**5**), and G (**7**) exhibited weak activity against α-glucosidase with IC_{50} values of 1.74−5.93 mM, and ampelomin A (**1**) showed moderate antibacterial activity with MIC_{90} values ranging from 202.4 to 1015.9 μM. A plausible polyketide biogenetic pathway is postulated for these compounds.

Glycosidases, the enzymes responsible for the hydrolysis of glycosidic bonds in poly- and oligosaccharides or glycoconjugates, are crucial in many metabolic pathways, including glycoprotein and glycolipid processing and carbohydrate digestion in the intestinal tract.[1] Inhibitors of glycosidases have potential for the treatment of various disorders and diseases such as diabetes, cancers, and AIDS.[1] Polyhydroxylated cyclohexanoids, a class of compounds closely related to carbasugars, are known to have activity as glycosidase inhibitors, and therefore they have received much attention in recent years. Though a number of known compounds have been isolated from plants and microbials and many more have been prepared through chemical synthesis,[2] the search for new polyhydroxylated cyclohexanoids with improved biological activities remains an active area of current research in natural products.

Ampelomyces sp. SC0307 is a soil-derived fungal strain possessing strong antibacterial activity. We recently reported the isolation of heteroatom-containing phenolics as the principle compounds responsible for antibacterial activity.[3] In continuing our study, we investigated the other metabolites of this fungus. The investigation led to the discovery of seven new polyoxygenated methyl cyclohexanoids, ampelomins A−G (**1**−**7**). We herein report the isolation, structure elucidation, biological activities, and plausible biogenetic pathway of these compounds.



Results and Discussion

The fermentation and extraction of the fungus have been described in our previous report.[3] The EtOAc and $CHCl_3$ extracts were fractionated by repeated column chromatography (CC) over silica gel, ODS, and Sephadex LH-20, preparative TLC, and HPLC to furnish seven new compounds, ampelomins A−G (**1**−**7**).

Ampelomin A (**1**), a yellowish oil, had a molecular formula of $C_7H_{10}O_2$ as deduced from the HRESIMS and ^{13}C NMR data. The ^{13}C NMR and DEPT spectra displayed seven signals, indicating the presence of a carbonyl group [δ 201.6 (C-1)], two olefinic methines [δ 153.5 (C-3), 128.4 (C-2)], an oxymethine [δ 67.2 (C-4)], an aliphatic methine [δ 40.1 (C-6)], a methylene [δ 41.2 (C-5)], and a methyl group [δ 14.8 (C-7)]. The ^1H NMR spectrum exhibited signals at δ 1.45 (3H, d, J = 6.5 Hz, H-7) for a methyl group, δ 4.64 (1H, ddt, J = 10.2, 4.8, 2.0 Hz, H-4) for an oxymethine, and δ 2.35−2.40 (2H, m, H-5eq and H-6) and 1.72 (1H, tdd, J = 12.2, 10.2, 2.0 Hz, H-5ax) for a methylene and a methine. In addition, the spectrum showed signals at δ 6.85 (1H, dt, J = 10.0, 2.0 Hz, H-2) and 5.95 (1H, dd, J = 10.0, 2.0 Hz, H-3) indicating the presence of a *cis* double bond. Interpretation of the COSY and HMQC spectra confirmed the presence of the functional groups noted above and allowed assignment of the gross structure as shown. The large proton coupling constants, $J_{4,5ax}$ = 10.2 Hz and $J_{5ax,6}$ = 12.2 Hz, showed an envelope conformation of the cyclohexenone ring in which both H-4 and H-6 were in pseudoaxial positions.

The absolute configuration of **1** was established using the modified Mosher's method.[4] Treatment of **1** with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetic acids (MTPA) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC-HCl) using catalytic DMAP[5] afforded (*R*)- and (*S*)-MTPA esters (**1a** and **1b**, respectively) (see Experimental Section). Analysis of the proton chemical shift differences ($\Delta\delta = \delta_S - \delta_R$) showed negative Δδ signs for H-3 (−0.11) and H-2 (−0.03) and positive signs for H-5ax (+0.09), H-5eq (+0.06), H-6 (+0.02), and H_3-7 (+0.02). This distribution of Δδ signs enabled assignment of the *R*-configuration to C-4.[4] Accordingly, the *S*-configuration of C-6 was assigned from the relative configuration. Therefore, ampelomin A was defined as (4*R*,6*S*)-4-hydroxy-6-methylcyclohex-2-enone (**1**).

Ampelomin B (**2**), a colorless oil, had a molecular formula of $C_7H_{12}O_3$, which was also determined from the HRESIMS and ^{13}C NMR data. The ^{13}C NMR (Table 2) and DEPT spectra exhibited seven carbon resonances, indicating the presence of a methyl group, a methylene, and five methines, of which four were oxygenated [δ 54.9 (C-2), 57.6 (C-3), 64.3 (C-1), and 68.5 (C-4)]. The upfield shifted oxymethine signals (δ 54.9 and 57.6) were assignable to an epoxy group. The ^1H NMR spectrum (Table 1) confirmed the presence of the functionalities indicated above. In addition, the spectrum displayed two exchangeable signals at δ 3.68 (1H, d, J = 7.6 Hz, 1-OH) and 4.18 (1H, d, J = 4.4 Hz, 4-OH), suggesting the presence of two hydroxy groups. Analysis of the COSY and HMQC spectra furnished full assignments of the ^1H and ^{13}C NMR data as shown in Tables 1 and 2 and led to basic structure **2**.

Since signals H-1 and H-4 appeared as complicated multiplets due to coupling with the protons of the geminal hydroxy group in

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Table 1. ^{1}H NMR (400 MHz) Data for Compounds **2**−**7**

position	**2**[a]	**2**[b]	**3**[c]	**3**[b]	**4**[d]	**5**[d]	**6**[d]	**7**[e]
1	4.05 m	4.63 td (5.4, 5.4, 3.7)	3.39 m	4.25 td (9.2, 4.2)	4.42 br s	4.31 br dd (5.8, 3.6)	4.04 ddd (11.4, 4.2, 3.2)	3.91 ddd (11.8, 9.2, 4.6)
2	3.21 t (3.7)	3.77 t (3.7)	3.23 m	4.06 td (9.2, 4.2)	4.61 dt (10.4, 4.4)	4.00 ddd (10.0, 5.8, 2.8)	4.46 br s	5.00 t (9.2)
3eq	3.13 dd (3.7, 1.8)	3.71 dd (3.7, 1.7)	1.70 dt (12.9, 3.8)	2.52 dt (12.7, 4.2)	2.36 dt (12.8, 4.4)	2.62−2.46 m	2.67 dt (13.2, 4.2)	
3ax			1.44 dt (12.9, 9.0)	2.27 dt (12.9, 9.2)	2.43 ddd (12.8, 10.4, 2.8)	2.62−2.46 m	1.86 ddd (13.2, 10.8, 2.2)	3.66 t (9.2)
4	3.89 m	4.35 dd (4.0, 1.7)	3.57 m	4.15 dt (9.2, 4.2)	4.23 br s	3.50 td (9.6, 5.9)	4.16 td (10.8, 4.2)	3.63 dd (9.2, 5.1)
5	1.89 m	2.34 m	1.85 m	2.36 m	2.39 m	2.35 m	1.74 m	2.18 m
6eq	1.18 ddd (13.8, 4.3, 3.2)	1.71 ddd (14.0, 5.4, 3.1)	1.59 ddd (13.4, 5.9, 4.2)	2.15 dt (13.4, 4.2)	1.98 dt (13.6, 4.0)	2.14 dt (14.0, 3.6)	1.98 dt (12.2, 4.2)	1.91 ddd (13.4, 4.6, 3.0)
6ax	1.57 ddd (13.8, 10.3, 5.8)	2.02 ddd (14.0, 9.9, 5.4)	1.23 ddd (13.4, 9.0, 4.2)	1.82 ddd (13.4, 9.2, 4.2)	2.08 ddd (13.6, 11.2, 2.4)	1.28 ddd (14.0, 12.2, 2.4)	2.10 td (12.2, 11.4)	1.67 ddd (13.4, 11.8, 4.6)
7	0.86 d (7.1)	1.14 d (7.1)	0.83 d (7.1)	1.20 d (7.1)	1.17 d (6.8)	1.23 d (6.6)	1.27 d (6.4)	1.03 d (7.4)

[a] Measured in acetone-d_6. Data for exchangeable protons: δ 3.68 (1H, d, J = 7.6 Hz, 1-OH) and 4.18 (1H, d, J = 4.4 Hz, 4-OH). [b] Measured in C_5D_5N−D_2O (5:1). [c] Measured in DMSO-d_6. Data for exchangeable protons: δ 4.46 (1H, d, J = 3.6 Hz, 1-OH), 4.67 (1H, d, J = 4.8 Hz, 2-OH), and 4.47 (1H, d, J = 5.0 Hz, 4-OH). [d] Measured in C_5D_5N. [e] Measured in CD_3OD. Data for 6-MSA moiety: δ 6.74 (2H, d, J = 8.0 Hz, H-3′ and H-5′), 7.23 (1H, t, J = 8.0 Hz, H-4′), and 2.53 (s, 6′-Me).

Table 2. ^{13}C NMR (100 MHz) Data for Compounds **2**−**7**[a]

position	**2**	**3**	**4**	**5**	**6**	**7**[b]
1	64.3	69.3	70.0	69.6	71.8	68.2
2	54.9	71.8	68.7	71.3	71.2	82.2
3	57.6	35.1	37.7	39.5	41.6	72.6
4	68.5	69.0	70.4	74.0	70.4	75.3
5	28.3	32.4	30.6	33.9	39.2	33.5
6	32.8	34.1	34.8	37.9	36.9	36.8
7	15.9	14.0	17.9	18.8	19.4	13.2

[a] Solvents: acetone-d_6 for **2**, DMSO-d_6 for **3**, C_5D_5N for **4**, **5**, and **6**, CD_3OD for **7**. [b] Data for 6-MSA moiety: δ 117.1 (C-1′), 160.9 (C-2′), 115.6 (C-3′), 133.9 (C-4′), 123.5 (C-5′), 141.4 (C-6′), 171.7 (C-7′), and 22.7 (6′-Me).

the ^{1}H NMR spectrum of **2** in acetone-d_6, it was quite difficult to define their coupling patterns. The ^{1}H NMR spectrum of **2** was remeasured in a mixture of C_5D_5N and D_2O (5:1) (Table 1). The remeasured ^{1}H NMR spectrum revealed the coupling constants, $J_{1,2} = J_{2,3} = 3.7$ Hz, $J_{3,4} = 1.7$ Hz, $J_{4,5} = 4.0$ Hz, $J_{5,6eq} = 3.1$ Hz, $J_{5,6ax} = 9.9$ Hz, and $J_{1,6eq} = J_{1,6ax} = 5.4$ Hz. In the NOESY spectrum of **2**, measured in the same mixture, key NOE interactions were observed between H-1/H-6ax, H-1/H-6eq, H-1/H-2, H-2/H-3, H-3/H-4, H-4/H-5, and H-4/H$_3$-7, while no correlations were found between H-5/H-6ax, H-5/H-1, and H-4/H-6ax. These findings showed a half-chair conformation for the cyclohexane ring in which 1-OH and 4-OH were pseudoaxial, 5-Me was equatorial, and H-1, H-2, and H-3 had a mutual *cis* relationship and a *trans* relationship to H-4.[6] Therefore, **2** was deduced to be (1*S**,2*S**,3*R**,4*S**,5*S**)-2,3-epoxy-5-methylcyclohexane-1,4-diol (**2**).

Ampelomins C−F (**3**−**6**), all obtained as white powders, had the same molecular formula, $C_7H_{14}O_3$, as determined from the HRESIMS and ^{13}C NMR data. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) showed that they all comprised a secondary methyl group, two methylenes, and four methines, of which three were oxygenated. By interpretation of COSY and HMQC, their basic structures were all derived to be 5-methylcyclohexane-1,2,4-triol. The structural differences were derived from configurational diversity, which was assigned by examination of the vicinal proton coupling constants in the ^{1}H NMR spectra. In the ^{1}H NMR spectrum of **3** in DMSO-d_6, the signals of H-1, H-2, and H-4 appeared as complicated multiplets. In order to provide coupling patterns of these signals, the ^{1}H NMR spectrum of **3** was remeasured in the mixture of C_5D_5N and D_2O (5:1). The remeasured ^{1}H NMR spectrum of **3** revealed axial−axial coupling constants ($J_{a,a} = 9.2$ Hz) between H-1/H-2, H-1/H-6ax, H-2/H-3ax, and H-3ax/H-4 and axial−equatorial or equatorial−equatorial coupling constants ($J_{a,e/e,e} = 4.2$ Hz) between H-1/H-6eq, H-2/H-3eq, H-3eq/H-4, H-4/H-5, H-5/H-6ax, and H-5/H-6eq. These values were consistent with

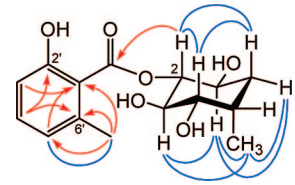
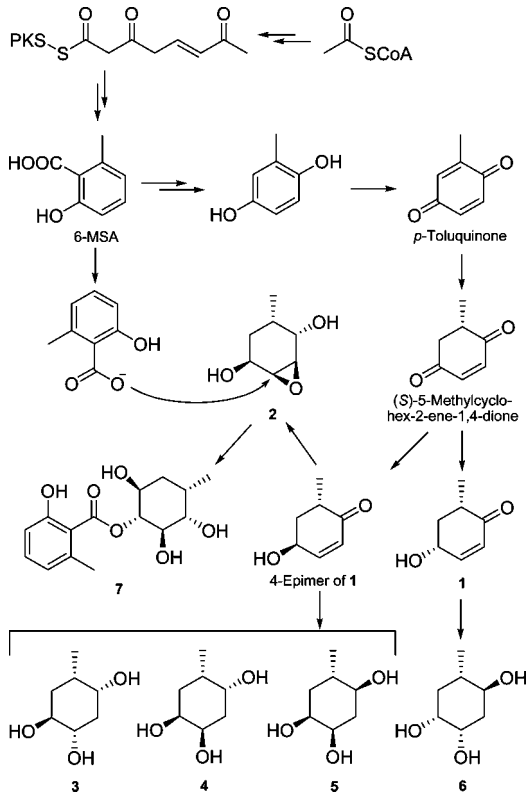


Figure 1. Key HMBC (arrows) and NOESY (curves) correlations of **7**.

a chair conformation for the cyclohexane ring in which the three hydroxy groups were in equatorial positions, while 5-Me was in the axial position. In the ^{1}H NMR spectrum of **4**, $J_{a,a}$ values between H-2/H-3ax (10.4 Hz) and H-5/H-6ax (11.2 Hz) and $J_{a,e/e,e}$ values (2.4−4.4 Hz) for other couplings were observed, indicating a chair form for the six-membered ring with 1-OH and 4-OH equatorial and 2-OH and 5-Me axial. For **5**, $J_{a,a}$ values (9.6−12.2 Hz) between H-2/H-3ax, H-3ax/H-4, H-4/H-5, and H-5/H-6ax and $J_{a,e/e,e}$ values (2.4−5.8 Hz) for other couplings were found, in accord with a chair form for the six-membered ring with 1-OH in the axial orientation and other substituents in equatorial orientations. For **6**, the $J_{a,a}$ values (10.8−12.2 Hz) found between H-1/H-6ax, H-3ax/H-4, H-4/H-5, and H-5/H-6ax and $J_{a,e/e,e}$ values (2.2−4.2 Hz) for other coupling constants showed that the cyclohexane ring adopted a chair conformation with 2-OH in the axial position and other substituents in equatorial positions. Therefore, the structures of ampelomins C−F were elucidated as (1*S**,2*S**,4*R**,5*S**)-5-methylcyclohexane-1,2,4-triol (**3**), (1*S**,2*R**,4*R**,5*S**)-5-methylcyclohexane-1,2,4-triol (**4**), (1*S**,2*R**,4*S**,5*S**)-5-methylcyclohexane-1,2,4-triol (**5**), and (1*R**,2*S**,4*S**,5*S**)-5-methylcyclohexane-1,2,4-triol (**6**), respectively.

Ampelomin G (**7**), a white solid, had a molecular formula of $C_{15}H_{20}O_6$ as determined from the HRESIMS and ^{13}C NMR data. The ^{1}H (Table 1), ^{13}C (Table 2), and DEPT NMR spectra showed the presence of a secondary methyl group, an aromatic methyl group [δ_H 2.53 (3H, s, 6′-Me); δ_C 22.7 (6′-Me)], a methylene, an aliphatic methine, four oxymethines, and an ester carbonyl group [δ_C 171.7 (C-7′)], as well as a 1,2,3-trisubstituted benzene ring [δ_H 7.23 (1H, t, J = 8.0 Hz, H-4′) and 6.74 (2H, d, J = 8.0 Hz, H-3′ and H-5′); δ_C 160.9 (C-2′), 141.4 (C-6′), 133.9 (C-4′), 123.5 (C-5′), 117.1 (C-1′), and 115.6 (C-3′)]. Interpretation of the COSY and HMQC spectra led to a partial structure, 5-methylcyclohexane-1,2,3,4-tetrol. The other functional groups were assembled into another partial structure, 6-methylsalicylic acid (6-MSA), from the HMBC spectrum (key correlations depicted in Figure 1), in which correlations were observed from the aromatic methyl protons at δ 2.53 to C-1′, C-5′, and C-6′ and from H-4′ to C-2′ and C-6′. The presence of the 6-methylsalicylic acid moiety was confirmed by alkaline

Scheme 1. Plausible Biogenetic Pathway for **1**−**7**



hydrolysis of **7**, which afforded 6-MSA, a metabolite previously obtained from this fungus.[3] The attachment of the 6-MSA moiety to C-2 was indicated by the downfield shifts of H-2 (δ 5.00) and C-2 (δ 82.2) as well as the presence of an HMBC correlation from H-2 to C-7′. The relative configuration was determined from the vicinal proton coupling constants in the ^{1}H NMR spectrum. The $J_{a,a}$ values H-1/H-2 (9.2 Hz), H-2/H-3 (9.2 Hz), H-3/H-4 (9.2 Hz), and H-6ax/H-1 (11.8 Hz) were observed, and the $J_{a,e/e,e}$ values H-4/H-5 (5.1), H-5/H-6eq (3.0), H-5/H-6ax (4.6 Hz), and H-1/H-6eq (4.6 Hz) were also observed, indicative of a chair conformation for the cyclohexane ring in which only the 5-Me substituent was in an axial position, while the other substituents were all in equatorial positions. The configuation was further supported by correlations in the NOESY spectrum (Figure 1). Thus, the structure of **7** was deduced as (1*R**,2*R**,3*S**,4*S**,6*S**)-2,3,6-trihydroxy-4-methylcyclohexyl 2-hydroxy-6-methylbenzoate (**7**).

The absolute configuration of **2**−**7** was not assigned in the present study. However, in the postulated biogenetic pathway (see next paragraph), **1**−**7** are derived from a common precursor, (*S*)-5-methylcyclohex-2-ene-1,4-dione. In consideration of this, the absolute configuration of **2**−**7** could be tentatively assigned as shown.

Polyoxygenated methyl cyclohexanoids of fungal origin,[7] such as theobroxide,[8] epoformin,[8] and terremutin,[9] have been proven to be biosynthetically derived from a polyketide pathway via a tetraketide intermediate, 6-MSA.[8,10] The co-occurrence of **1**−**7** and 6-MSA in this *Ampelomyces* sp. strongly suggests that these metabolites are produced via a similar biosynthetic pathway. By inspection of the structures of **1**−**7**, it is clear that these metabolites are chemically related (Scheme 1); that is, **7** might be derived from **2** through reaction with 6-MSA via an S_N2 mechanism, **2** might be generated from the 4-epimer of **1** by epoxidation and reduction, and **3**−**6** could be transformed from **1** or its 4-epimer via hydration in a mechanism similar to that for α,β-unsaturated ketones[11] followed by reduction. Thus, a plausible but speculative biogenetic pathway for **1**−**7** is postulated as shown in Scheme 1. The uniqueness of this pathway, compared with the previously proposed routes, is that *p*-toluquinone may undergo partial hydrogenation, leading to (*S*)-5-methylcyclohex-2-ene-1,4-dione, the key precursor for **1**−**7**, instead of a reaction involving epoxide precursors.[8−10]

Table 3. Antibacterial Activities of **1**, Determined by MABA (μM)

	1		ceftazidime	
bacteria	$MIC_{50} \pm SD$	$MIC_{90} \pm SD$	$MIC_{50} \pm SD$	$MIC_{90} \pm SD$
S. aureus	54.9 ± 5.4	234.2 ± 8.7	3.11 ± 0.16	14.65 ± 0.37
E. coli	89.9 ± 4.8	265.1 ± 16.3	0.37 ± 0.15	0.46 ± 0.09
P. vulgaris	51.6 ± 4.8	202.4 ± 4.8	0.18 ± 0.11	0.21 ± 0.01
P. aeruginosa	63.5 ± 0.8	1015.9 ± 19.8	0.73 ± 0.07	3.94 ± 0.16

The closely related carbasugar structures of these compounds, among which **3**−**6** could be regarded as 2,6-deoxy-5a-carbasugars and **2** and **7** as 6-deoxy-5a-carbasugars, inspired us to evaluate their glycosidase inhibitory activities. In the evaluation **1**, **3**, **5**, and **7** exhibited activity against α-glucosidase with IC_{50} values of 1.74−5.93 mM, much weaker than acarbose (IC_{50} = 0.03 mM), while **2**, **4**, and **6** were not active toward α-glucosidase. All compounds were inactive against β-glucosidase and α-rhamnosidase at concentrations of 10 mM and lower. It was interesting that the active methylcyclohexanes (**3**, **5**, and **7**) all possess an equatorial 2-*O*-substituent, differing from the inactive compounds (**2**, **4**, and **6**), which all have an axial or pseudoaxial 2-*O*-substituent. At present, there is no explanation for such an SAR regarding α-glucosidase inhibition. The antibacterial activity of these compounds against *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, and *Pseudomonas aeruginosa* was also evaluated, and only **1** showed activity, with MIC_{50} values of 51.6−89.7 μM and MIC_{90} values ranging from 202.4 to 1015.9 μM, less potent than ceftazidime (Table 3).

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a Perkin-Elmer 343 spectropolarimeter. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), and 2D NMR spectra were recorded on a Bruker DRX-400 instrument with the residual solvent peak as reference. HRESIMS data were obtained on a Bruker Bio TOF IIIQ mass spectrometer. ESIMS data were collected on a MDS SCIEX API 2000 LC/MS/MS instrument. Preparative HPLC was run with a Shimazu LC-6AD pump and a Shimazu RID-10A refractive index detector using a XTerra prep MS C_{18} column (10 μm, 300 × 19 mm). Preparative TLC was performed on precoated silica gel plates (GF_{254}, Qingdao Marine Chemical Ltd., Qingdao, China, 0.25 mm thickness) with detection under fluorescent light (λ = 254 nm). For column chromatography, silica gel 60 (100−200 mesh, Qingdao Marine Chemical Ltd., Qingdao, China), Develosil ODS (10 μm, Nomura Chemical Co. Ltd., Japan), and Sephadex LH-20 were used.

Producing Fungus and Fermentation. The producing fungus, *Ampelomyces* sp. SC0307, was isolated from a soil sample collected in the Dinghu Mountain Biosphere Reserve, Guangdong, China, in January 2002.[3] The fermentation process was described in our previous report.[3] Briefly, the seed culture (3000 mL) was prepared by growing the fungus in YMG broth on a rotary shaker for 3 days in the dark at 28 °C, 120 rpm. The culture were transferred into 30 5000 mL Erlenmeyer flasks containing 1000 mL of YMG medium and 550 g of wheat grains, and the flasks were incubated for 7 days in the stationary phase in the dark at 28 °C.

Extraction and Isolation. The obtained mycelial solid culture (the medium upon which the mycelia were grown) was extracted with 95% EtOH, and the resultant extract was partitioned using sequential extraction with petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH.[3] The EtOAc-soluble extract (36 g) was separated into 10 fractions by Si gel column chromatography (CC) using $CHCl_3$−MeOH mixtures of increasing polarity (100:0 to 60:40). Fraction 5, obtained on elution with $CHCl_3$−MeOH (95:5), was further chromatographed on a Si gel column using $CHCl_3$−MeOH (95:5 to 80:20) and resulted in the collection of 40 subfractions of 35 mL each. Subfractions 11−14, obtained on elution with $CHCl_3$−MeOH (95:5), were combined on the basis of TLC analysis and purified by Sephadex LH-20 CC using MeOH

to afford compound **2** (20 mg). Fraction 9, obtained on elution with $CHCl_3$−MeOH (9:1), was subjected to Si gel CC using petroleum ether−acetone (6:4−4:6) and resulted in the collection of 27 subfractions of 100 mL each. Subfractions 10 and 11, obtained on elution with petroleum ether−acetone (55:45), were combined and applied to Sephadex LH-20 CC using MeOH, followed by preparative HPLC using 5% aqueous MeOH to afford **3** (15 mg) (t_R = 19.98 min) and **4** (50 mg) (t_R = 24.13 min). Fraction 10, obtained on elution with $CHCl_3$−MeOH (9:1), was subjected to Si gel CC using petroleum ether−acetone (6:4−4:6) and resulted in the collection of 27 subfractions of 10 mL each. Subfraction 8, obtained on elution with petroleum ether−acetone (55:45), was purified by preparative HPLC using 5% aqueous MeOH to afford **6** (9 mg) (t_R = 20.30 min).

The $CHCl_3$-soluble extract (28 g) was subjected to passage over a Si gel column, eluted with petroleum ether−acetone mixtures in a step gradient of increasing polarity (95:5 to 50:50) to obtain 10 fractions. Fractions 4, 7, and 10, obtained by elution with petroleum ether−acetone (9:1, 6:4, and 5:5, respectively), were applied to further separation. Fraction 4 was chromatographed on an ODS column using 60% aqueous MeOH (6:4), followed by purification with Sephadex LH-20 CC using MeOH to afford **1** (20 mg). Fraction 7 was chromatographed on an ODS column and eluted with 30% MeOH to obtain 59 subfractions of 10 mL each. Subfractions 22−30 were combined and purified by Sephadex LH-20 CC using MeOH, followed by preparative TLC ($CHCl_3$−MeOH, 9:1, R_f = 0.55), to afford **7** (12 mg). Fraction 10 was chromatographed on a Si gel column using $CHCl_3$−MeOH mixtures of increasing polarity (90:10 to 60:40) to obtain 30 subfractions of 35 mL each. Subfraction 4 was purified by Sephadex LH-20 CC using MeOH, followed by HPLC using 5% MeOH, to afford **5** (20 mg) (t_R =33.25 min).

Ampelomin A (1): yellowish oil; $[\alpha]_D^{20}$ +50.1 (*c* 1.6, MeOH); positive ESIMS *m/z* 253 $[2M + H]^+$, 165 $[M + K]^+$, 149 $[M + Na]^+$, 127 $[M + H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.85 (1H, dt, J = 10.0, 2.0 Hz, H-2), 5.95 (1H, dd, J = 10.0, 2.0 Hz, H-3), 4.64 (1H, ddt, J = 10.2, 4.8, 2.0 Hz, H-4), 2.35−2.40 (2H, m, H-5eq and H-6), 1.72 (1H, tdd, J = 12.2, 10.2, 2.0 Hz, H-5ax), 1.45 (3H, d, J = 6.5 Hz, H_3-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.6 (C-1), 128.4 (C-2), 153.5 (C-3), 67.2 (C-4), 41.2 (C-5), 40.1 (C-6), 14.8 (C-7); HRESIMS *m/z* 127.0758 $[M + H]^+$ (calcd for $C_7H_{11}O_2$, 127.0754).

Ampelomin B (2): colorless oil; $[\alpha]_D^{20}$ +90.6 (*c* 0.415, MeOH); positive ESIMS *m/z* 311 $[2M + Na]^+$, 183 $[M + K]^+$, 167 $[M + Na]^+$; ^{1}H NMR (400 MHz) see Table 1; ^{13}C NMR (100 MHz) see Table 2; HRESIMS *m/z* 167.0676 $[M + Na]^+$ (calcd for $C_7H_{12}O_3Na$, 167.0679).

Ampelomin C (3): white solid; $[\alpha]_D^{20}$ −15.3 (*c* 0.74, MeOH); positive ESIMS *m/z* 315 $[2M + Na]^+$, 293 $[2M + H]^+$, 169 $[M + Na]^+$, 147 $[M + H]^+$; ^{1}H NMR (400 MHz) see Table 1; ^{13}C NMR (100 MHz) see Table 2; HRESIMS *m/z* 169.0848 $[M + Na]^+$ (calcd for $C_7H_{14}O_3Na$, 169.0835).

Ampelomin D (4): white solid; $[\alpha]_D^{20}$ −5.9 (*c* 0.22, MeOH); positive ESIMS *m/z* 315 $[2M + Na]^+$, 169 $[M + Na]^+$; ^{1}H NMR (400 MHz) see Table 1; ^{13}C NMR (100 MHz) see Table 2; HRESIMS *m/z* 169.0841 $[M + Na]^+$ (calcd for $C_7H_{14}O_3Na$, 169.0835).

Ampelomin E (5): white solid; $[\alpha]_D^{20}$ −46.7 (*c* 0.67, MeOH); positive ESIMS *m/z* 315 $[2M + Na]^+$, 169 $[M + Na]^+$; ^{1}H NMR (400 MHz) see Table 1; ^{13}C NMR (100 MHz) see Table 2; HRESIMS *m/z* 169.0820 $[M + Na]^+$ (calcd for $C_7H_{14}O_3Na$, 169.0835).

Ampelomin F (6): white solid; $[\alpha]_D^{20}$ +37.6 (*c* 0.5, MeOH); positive ESIMS *m/z* 315 $[2M + Na]^+$, 169 $[M + Na]^+$; ^{1}H NMR (400 MHz) see Table 1; ^{13}C NMR (100 MHz) see Table 2; HRESIMS *m/z* 145.0864 $[M - H]^-$ (calcd for $C_7H_{13}O_3$, 145.0870).

Ampelomin G (7): white solid; $[\alpha]_D^{20}$ −17.0 (*c* 0.73, MeOH); positive ESIMS *m/z* 319 $[M + Na]^+$; ^{1}H NMR (400 MHz) see Table 1; ^{13}C NMR (100 MHz) see Table 2; HRESIMS *m/z* 319.1149 $[M + Na]^+$ (calcd for $C_{15}H_{20}O_6Na$, 319.1152).

Preparation of the (*R*)-MTPA and (*S*)-MPTA Esters of 1. A solution of **1** (3.0 mg, 0.024 mmol) in CH_2Cl_2 (2.0 mL) was treated with (*R*)-MTPA (28.1 mg, 0.12 mmol) in the presence of EDC-HCl (23.0 mg, 0.12 mmol) and 4-DMAP (14.6 mg, 0.12 mmol), and the mixture was stirred at room temperature (25 °C) for 7 days. The reaction mixture was poured into ice−water, and the mixture was extracted with $CHCl_3$. The $CHCl_3$ extract was successively washed with 5% HCl, saturated $NaHCO_3$, and brine, dried over $MgSO_4$, and filtered. Evaporation of the solvent furnished a residue, which was purified by Si gel CC (1 g, $CHCl_3$) to give the (*R*)-MTPA ester (0.6 mg). Through a similar procedure, the (*S*)-MTPA ester (0.6 mg) was prepared from **1** (3.0 mg) by the use of (*S*)-MTPA (28.1 mg), EDC-HCl (23.0 mg), and 4-DMAP (14.6 mg).

(*R*)-MTPA Ester of 1 (1a): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.510 (2H, m, Ar−H), 7.410 (3H, m, Ar−H), 6.770 (1H, dd, J = 10.2, 2.0 Hz, H-2), 6.066 (1H, dd, J = 10.2, 2.0 Hz, H-3), 5.891 (1H, ddd, J = 7.6, 5.2, 2.8 Hz, H-4), 3.550 (3H, s, OCH_3), 2.480 (1H, m, H-6), 2.440(1H, m, H-5eq), 1.837 (1H, dddd, J = 12.2, 7.6, 5.2, 2.8 Hz, H-5ax), 1.151 (3H, d, J = 6.8 Hz, H_3-7).

(*S*)-MTPA Ester 1 (1b): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.510 (2H, m, Ar−H), 7.420 (3H, m, Ar−H), 6.663 (1H, dd, J = 10.2, 2.0 Hz, H-2), 6.034 (1H, dd, J = 10.2, 2.0 Hz, H-3), 5.900 (1H, ddd, J = 7.6, 5.2, 2.8 Hz, H-4), 3.550 (3H, s, OCH_3), 2.501 (2H, m, H-5eq and H-6), 1.925 (1H, dddd, J = 7.6, 5.2, 2.8 Hz, H-5ax), 1.175 (3H, d, J = 6.8 Hz, H_3-7).

Glycosidase Inhibition Assay. The enzymes of α-glucosidase from baker's yeast, β-glucosidase from almonds, and naringinase from *Penicillium decumbens*, as well as the substrates of *p*-nitrophenyl α-D-glucopyranoside, *p*-nitrophenyl β-D-glucopyranoside, and *p*-nitrophenyl α-L-rhamnopyranoside, were purchased from Sigma Chemical Company. Other chemicals were purchased from native companies. The glycosidase inhibition assay was performed according to the reported methods[12−14] with slight modification. As for the α-glucosidase inhibition assay, 50 μL of 0.2 U/mL enzyme in 0.2 M phosphate buffer (pH 6.8) and 50 μL of compound solution in the same buffer were incubated in 96-well plates at 37 °C for 30 min. Then, 50 μL of 2.5 mM *p*-nitrophenyl α-D-glucopyranoside in 0.2 M phosphate buffer was added, and the plate was incubated at 37 °C for another 5 min. The reaction was quenched by the addition of 0.1 M Na_2CO_3 solution (0.1 mL). Acarbose was tested as a positive control. Before and after reaction, absorbance readings were recorded at 405 nm by microplate reader (Genios) and compared to a negative control, which had 50 μL of buffer solution in place of compound solution to calculate percentage inhibition. This procedure was also used for β-glucosidase and α-rhamnosidase inhibition assays. β-Glucosidase inhibition was evaluated using *p*-nitrophenyl β-D-glucopyranoside as a substrate, citrate-phosphate buffer (pH 5.0) as a reaction buffer, and borate buffer (pH 9.0) as an alkali solution to stop the reaction. α-Rhamnosidase inhibition was assayed using naringinase as an enzyme, *p*-nitrophenyl α-L-rhamnopyranoside as a substrate, citrate-phosphate buffer (pH 5.0) as a reaction buffer, and 0.4 M NaOH as an alkali solution to stop the reaction. Glycosidase inhibitory activity was expressed as IC_{50} values. The IC_{50} values (IC_{50} ± SD, mM) against α-glucosidase were determined as 3.59 ± 0.008 (**1**), 6.12 ± 0.006 (**3**), 5.93 ± 0.002 (**5**), 1.74 ± 0.004 (**7**), >10 (**2**, **4**, and **6**), and 0.03 ± 0.003 (acarbose). The values of all test compounds against β-glucosidase and α-rhamnosidase were more than 10 mM.

Antibacterial Activity. The antibacterial activity against *S. aureus*, *E. coli*, *P. vulgaris*, and *P. aeruginosa* was evaluated by microplate Alamar Blue assay as described in our previous report.[3] Briefly, serial 2-fold dilutions of compounds **1**−**7** and ceftazidime were made in DMSO, and the test bacteria at a concentration of 2 × 10^5 cfu/mL were prepared in MHB medium supplemented with 8% Alamar Blue. In a 96-well plate, 78 μL of each test organism and 2 μL each of the compound dilutions were mixed and incubated at 32 °C until growth control wells developed the growth (pink color). The fluorescence intensity was then measured using a plate reader (Synergy HT, Bio-Tek, Winooski, VT) with excitation at 530 nm and emission at 590 nm. The percentage inhibition of each compound dilution was calculated. All tests were run in triplicate and averaged. MIC_{50} and MIC_{90} values (Table 3), defined as the concentrations required to reduce bacterial growth by 50% and 90%, respectively, were obtained by interpolation of concentration−inhibition curves.

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