Ymf 1029A−E, Preussomerin Analogues from the Fresh-Water-Derived Fungus YMF 1.01029

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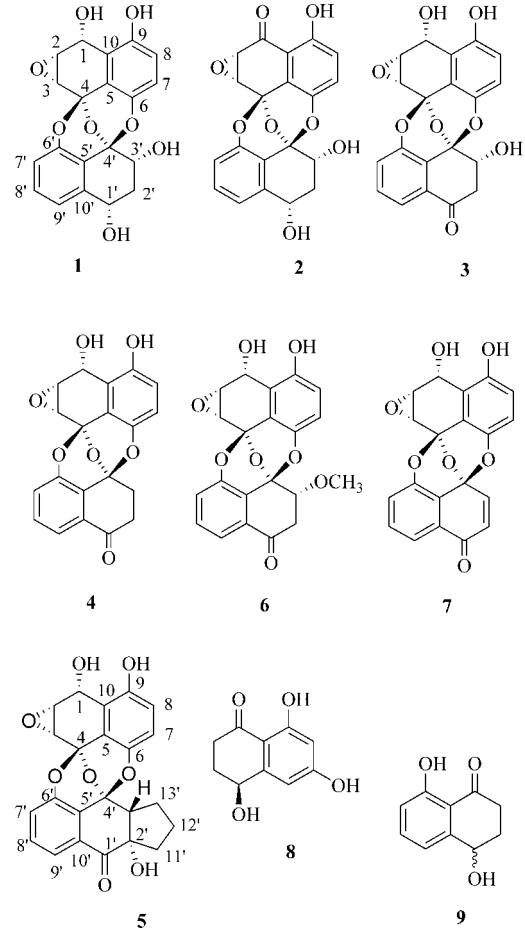
Five new preussomerin analogues, ymf 1029A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**), were isolated from the liquid cultures of an unidentified freshwater fungus YMF 1.01029, along with four known compounds, preussomerin C (**6**), preussomerin D (**7**), (4*RS*)- 4,8-dihydroxy-3,4-dihydronaphthalen-1(2*H*)-one (**8**), and 4,6,8-trihydroxy-3,4-dihydronaphthalen-1(2*H*)-one (**9**). The structures of new metabolites were determined by analysis of NMR and MS data and by analogy with the data for the known bis-spirobisnaphthalene preussomerins. *In vitro* immersion experiments showed that these metabolites displayed weak nematicidal activity against *Bursaphelenchus xylophilus*, while compound **7** was the most potent. This is the first report of these compounds, which antagonize the *Bursaphelenchus xylophilus* nematode.

Among the variety of biosynthetically related unusual bisnaphthospiroketal compounds appearing in the literature,[1–4] the most notable are the bis-spiroketal-containing preussomerins. These preussomerins have been reported to possess antibacterial/antifungal/antiplasmodial activities.[5–8] In addition, they contain ras farnesyl-protein transferase inhibitory activities.[8] During our search for bioactive natural products, we found that the extract of cultural filtrates of the fresh-water-derived fungus YMF1.01029 exhibit *in vitro* activity against *Bursaphelenchus xylophilus,* a plant-parasitic and fungal-feeding nematode that causes multimillion dollar losses to pine forests, especially in several Asian countries.[9,10] The observed nematocidal activities prompted us to analyze the chemical constituents of the culture filtrates. Our analyses identified five new preussomerin analogues, which we have designated as ymf 1029A−E (**1**−**5**). Aside from these five new compounds, we also identified four other compounds from this fungus with chemical structures identical to those reported elsewhere: preussomerin C (**6**), preussomerin D (**7**), (4*RS*)-4,8-dihydroxy-3,4-dihydronaphthalen-1(2*H*)-one (**8**), and 4,6,8-trihydroxy-3,4-dihydronaphthalen-1(2*H*)-one (**9**). In this paper, we describe the isolation, structure elucidation, and biological activity of metabolites **1**−**9**.



Results and Discussion

The unidentified freshwater fungus YMF1.01029 was fermented in potato dextrose broth (300 × 200 mL; 60 L). The EtOAc extract of the cultural filtrate was subjected to series of chromatographic separations to furnish five new bis-spirobisnaphthalene metabolites, ymf 1029A−E (**1**−**5**), and four related but known metabolites. The structures of the known compounds were established as two bis-spirobisnaphthalenes, preussomerin C (**6**)[6] and preussomerin D (**7**),[6] as well as two naphthalenones, 4,6,8-trihydroxy-3,4-dihydronaphthalen-1(2*H*)-one (**8**)[11,12] and 4,8-dihydroxy-3,4-dihydronaphthalen-1(2*H*)-one (**9**),[13] by comparing their spectroscopic data with those in the literature.

By analyzing the NMR data, we found that ymf 1029A−E (**1**−**5**) are new members of natural compounds in the preussomerin family. The general characteristics of this class of compounds include two unsaturated decalin units connected via three oxygen bridges through two spiroketal carbons located in each of the upper and lower decalin units resonating between δ_C 90 and 100. The NMR spectroscopic data for the 1,2,3-trisubstituted aromatic ring, the 1,2,3,4-tetrasubstituted aromatic ring, and the bridging carbon atom of compounds **1**−**5** were virtually identical. As a result, the structural elucidation of these metabolites focused mainly on establishing the identities of the remaining portions of the molecules.

The molecular formula of ymf 1029A (**1**) was determined to be $C_{20}H_{16}O_8$ (13 degrees of unsaturation) by HRTOFMS analysis (*m*/*z* 407.0738 $[M + Na]^+$, calcd, 407.0742), and this conclusion was supported by the ^1H and ^{13}C NMR data. Compound **1** had characteristic bis-spirobisnaphthalene NMR signals due to one

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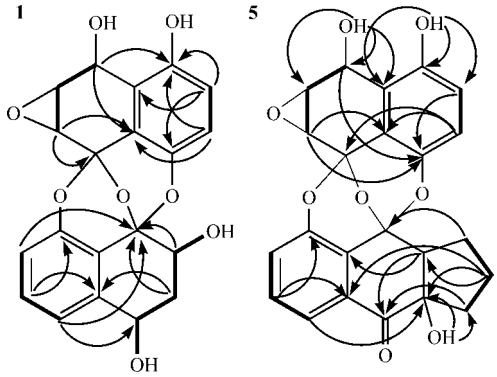

Figure 1. Key HMBC and COSY correlations for compounds **1** and **5**.

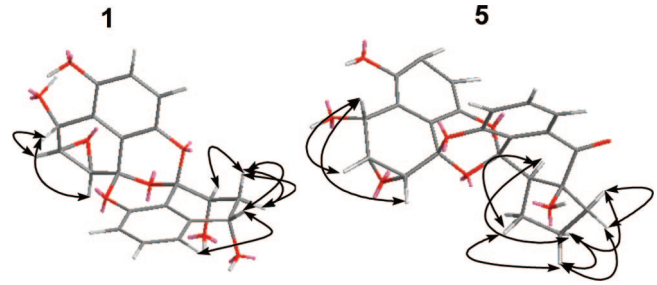

Figure 2. Calculated conformation by MM2 and key NOESY correlations for compounds **1** and **5**.

oxygenated 1,2,3,4-tetrasubstituted aromatic ring [δ_H 6.77 (d, J = 8.8 Hz, H-8) and 6.69 (d, J = 8.8 Hz, H-7); δ_C 152.4(C-9), 144.5 (C-6), 120.8 (C-8), 117.8 (C-7), 116.8 (C-10), and 115.9 (C-5)], one oxygenated 1,2,3-trisubstituted aromatic ring [δ_H 6.73 (d, J = 7.9 Hz, H-7′), 7.30 (t, J = 7.9 Hz, H-8′), and 7.19 (d, J = 7.6 Hz, H-9′); δ_C 151.9 (C-6′), 142.4 (C-10′), 131.6 (C-8′), 121.6 (C-9′), 117.7 (C-5′), and 115.2 (C-7′)], one phenolic OH group [δ_H 9.21 (brs, *H*O-9)], and two spiroketal quaternary carbons [δ_C 96.2 (C-4) and 95.9 (C-4′)]. In addition, the ^{1}H and ^{13}C NMR spectra showed signals for two epoxide-bearing methines [δ_H 3.92 (d, J = 4.6 Hz, H-3) and 3.87 (dd, J = 3.6, 1.0 Hz, H-2); δ_C 53.5 (C-3) and 53.6 (C-2)], three oxygenated methines [δ_H 5.60 (brs, H-1), 4.45 (brs, H-3′), 4.89 (brs, H-1′), 4.73 (brs, *H*O-3′), and 4.35 (brs, *H*O-1′); δ_C 69.8 (C-1), 69.3 (C-3′), and 65.7 (C-1′)], and one methylene [δ_H 2.98 (m, H-2′α) and 2.16 (t, J = 4.1 Hz, H-2′β); δ_C 36.8]. In the ^{1}H−^{1}H COSY spectrum in acetone-d_6, homonuclear coupling correlations of H-1 with H-2, H-2 with H-3, H-1′ with H-2′, and H-2′ with H-3′, as well as correlations of the hydroxyl protons with their vicinal protons, indicated unambiguously the presence of two isolated proton spin systems corresponding to the C-1−C-3 and HO-C-1′−C-3′-OH subunits of structure **1** (Figure 1). In the HMBC spectrum, the correlations of H-1 with C-10, C-9, C-8, C-6, C-5, C-3, and C-2, H-2 with C-9, and H-3 with C-4 and C-5 confirmed that C-1 and C-3 of the subunit C-1−C-3 were attached to C-10 and to C-4, respectively, while C-1′ and C-3′ of the subunit HO-C-1′−C-3′-OH were respectively bonded to C-10′ and to C-4′ on the basis of the other correlations of H-9′ with C-1′ and of H-2′, H-3′, H-7′, and H-9′ with C-4′ (Figure 1). The above spectral evidence led to the establishment of the planar structure of **1**. The absolute configurations at C-1, C-2, C-3, C-1′, and C-3′ were presumed to be the same as for the known preussomerins on the basis of the nearly identical NMR data of corresponding structural parts between ymf 1029A and the known preussomerins A−L,[5–7,14] the absolute stereochemistries of which were originally assigned on the basis of X-ray crystallography and chemical degradation experiments conducted on preussomerin A.[5,6] This assignment was also supported by the observed NOESY correlations of H-1/H-2, H-2/H-3, H-1′/H-9′, H-1′/βH-2′, and βH-2′/H-3′, as shown in **1** (Figure 2), which was generated by MM2 calculation.[15]

The HRTOFMS spectrum of ymf 1029B (**2**) showed an [M + Na]$^+$ ion at m/z 405.0585 (calcd, 405.0586), corresponding to the molecular formula $C_{20}H_{14}O_8Na$, which indicated that it had one more degree of unsaturation than compound **1**. Analysis of ^{1}H and ^{13}C NMR data of **2** revealed the presence of an isolated 1,2-disubstituted epoxide, two secondary oxomethines, one aliphatic methylene, and two aromatic rings. These proton spin systems and the presence of a highly chelated phenolic OH group at δ_H 10.66 ppm and a carbonyl signal at δ_C 197.1 ppm suggest that ymf 1029B (**2**) is closely related to ymf 1029A (**1**) and differs from **1** only in the oxidation state at C-1. Again, the structure of ymf 1029B was confirmed through a parallel set of NMR experiments (Tables 1 and 2).

Ymf 1029C (**3**) had the molecular formula $C_{20}H_{14}O_8$ according to HRTOFMS (m/z 405.0590 [M + Na]$^+$). This compound showed ^{1}H and ^{13}C NMR spectroscopic data similar to those of preussomerin C (**6**), except for the absence of the signal due to the methoxy group on C-3′ and the appearance of a hydroxyl proton at δ_H 5.14 ppm. On the basis of these data, compound **3** was readily identified as the 3′-*O*-demethyl analogue of preussomerin C. As a result, the H-3′ signal in **3** was shifted upfield to δ_H 4.75 ppm (δ_H 5.62 ppm in **6**) and the C-3′ signal in **3** moved upfield significantly compared with that of **6** (δ_C 70.9 vs δ_C 80.1 ppm). Detailed analysis of the NMR spectrum (^{1}H, ^{13}C, DEPTs, COSY, HMQC, and HMBC) led to the full assignment of proton and carbon atoms (Tables 1 and 2), and the structure depicted as **3** was confirmed. Likewise, the stereochemistry of ymf 1029C was deduced as shown in **3** by comparison of its NMR data with those of **6**, whose stereochemistry has been previously elucidated.[6]

The HRESIMS spectrum of ymf 1029D (**4**) showed an [M + Na]$^+$ ion at m/z 389.0653 (calcd, 389.0648), corresponding to the molecular formula $C_{20}H_{14}O_7Na$, which indicated that it had two additional protons compared to preussomerin D (**7**). The ^{1}H and ^{13}C NMR spectrum (Table 1) revealed that the trisubstituted and tetrasubstituted aromatic rings were intact, as well as an α-hydroxy epoxide, a conjugated carbonyl, and ketal functionalities as found in **7**. However, the ^{1}H NMR spectrum (Table 1) of **4** was devoid of the two doublets (δ_H 7.43 and 6.64) assigned to *cis*-olefinic protons H-3′ and H-2′ of preussomerin D and instead displayed four self-coupled aliphatic protons [δ_H 3.38 (m, H_2-3′), 3.35 (m, αH-2′), and 2.74 (m, βH-2′)] in the upfield region of the spectrum, which were respectively assigned to the H-3′ and H-2′ protons. The ^{13}C NMR spectrum contained signals for two upfield methylene carbons (δ_C 38.7 and 39.8) and only 12 sp^2-hybridized carbons. These data disclosed that the only difference between **4** and preussomerin D was saturation of the C-2′, C-3′ double bond, revealing the structure of Ymf 1029D as **4**. The proton and carbon assignments for **4** were determined through HMQC and HMBC experiments.

The NMR and HRESIMS data for ymf 1029E (**5**) revealed its molecular formula as $C_{23}H_{18}O_8$ (m/z 445.0902 [M + Na]$^+$, calcd, 445.0899), which indicated 15 degrees of unsaturation. Aside from the characteristic bis-spirobisnaphthalene NMR signals due to one oxygenated 1,2,3,4-tetrasubstituted aromatic ring, one oxygenated 1,2,3-trisubstituted aromatic ring, one α-hydroxy epoxide, and two spiroketal quaternary carbons, the ^{1}H and ^{13}C NMR spectra showed the presence of signals due to one conjugated carbonyl carbon at δ_C 205.9 (C-1′), one oxygenated quaternary carbon at δ_C 72.4 (C-2′), one methine [δ_C 40.2 (C-3′) and δ_H 3.04 (m, H-3′)], and three methylenes [δ_C 39.1 (C-13′), 37.2 (C-12′), 60.7 (C-11′); δ_H 2.62 (d, J = 4.0 Hz, H_2-13′), 2.50 (dd, J = 13.1, 4.0 Hz, βH-12′), 3.25 (dt, J = 13.1, 4.0 Hz, αH-12′), 2.30 (dd, J = 13.1, 4.0 Hz, βH-11′), 2.84 (m, αH-11′)]. These data, combined with the COSY correlations of H-3′ with H-13′, H-13′ with H-12′, and H-12′ with

Table 1. ^{1}H NMR Chemical Shift Assignments (δ) and Coupling Data of Compounds **1**−**7** (CD_3COCD_3)

C	1	2	3	4	5	6	7
1	5.60 (br s)		5.66 (br s)	5.61 (d, 5.3)	5.57 (d, 5.3)	5.61 (d, 5.3)	5.66 (br s)
2	3.87 (dd, 3.6, 1.0)	3.95 (dd, 4.5, 1.1)	3.92 (dd, 4.5, 1.8)	3.89 (dd, 4.5, 0.9)	3.84 (dd, 4.5, 1.8)	3.90 (dd, 4.4, 1.8)	3.95 (dd, 4.6, 1.3)
3	3.92 (d, 4.6)	4.27 (d, 4.6)	3.99 (d, 4.5)	3.98 (d, 4.6)	3.91 (d, 4.5)	4.03 (d, 4.5)	4.05 (d, 4.6)
7	6.69 (d, 8.8)	6.89 (d, 8.9)	6.74 (d, 9.0)	6.76 (d, 8.8)	6.66 (d, 9.0)	6.77 (d, 9.0)	6.74 (d, 9.3)
8	6.77 (d, 8.8)	6.97 (d, 9.0)	6.80 (d, 9.0)	6.68 (d, 8.8)	6.72 (d, 9.1)	6.87 (d, 9.0)	6.80 (d, 9.3)
9-OH	9.21 (s)	10.66 (s)	9.25 (s)	9.21 (s)	9.12 (s)	9.11 (s)	9.20 (s)
1′	4.89 (br s)	4.86 (br s)					
1′-OH	4.35 (br s)	4.33 (br s)					
2′	2.16 (βH, t, 4.1) 2.98 (αH, m)	2.12 (βH, t, 4.2) 3.17 (αH, m)	2.93 (βH, dd, 18.0, 1.5) 3.43 (αH, dd, 18.0, 1.5)	2.74 (βH, m) 3.35 (αH, m)		3.02 (βH, dd, 18.1, 1.4) 3.38 (αH, dd, 18.1, 1.4)	6.64 (d, 10.0)
2′-OH					5.07 (s)		
3′	4.45 (br s)	4.48 (br s)	4.75 (d, 3.1))	3.38 (m)	3.04 (m)	5.62 (br s)	7.43 (d, 10.0)
3′-OH	4.73 (br s)	4.78 (br s)	5.14 (s)				
3′-OMe						3.49 (s)	
7′	6.73 (d, 7.9)	6.71 (d, 8.3)	7.14 (d, 8.2)	7.17 (d, 8.1)	6.59 (d, 8.0)	7.13 (d, 8.1)	7.15 (d, 8.1)
8′	7.30 (t, 7.9, 7.9)	7.25 (t, 8.0, 7.8)	7.46 (t, 8.1, 7.7)	7.49 (t, 8.0, 7.8)	7.26 (t, 8.0, 7.7)	7.44 (t, 8.0, 7.9)	7.55 (t, 8.0, 7.9)
9′	7.19 (d, 7.6)	7.15 (d, 7.9)	7.58 (d, 7.7)	7.54 (d, 7.8)	7.21 (d, 7.7)	7.60 (d, 7.9)	7.57 (d, 7.7)
11′					2.30 (βH, dd, 13.1, 4.0) 2.84 (αH, m)		
12′					2.50 (βH, dd, 13.1, 4.0) 3.25 (αH, dt, 13.1, 4.0)		
13′					2.62 (d, 4.0)		

Table 2. ^{13}C NMR Chemical Shift Assignments (δ) of Compounds **1**−**7** (CD_3COCD_3)

C	1	2	3	4	5	6	7
1	69.8 (CH)	197.1 (C)	69.3 (CH)	69.3 (CH)	69.0 (CH)	69.2 (CH)	69.5 (CH)
2	53.6 (CH)	53.1 (CH)	53.2 (CH)	53.1 (CH)	53.2 (CH)	53.1 (CH)	53.6 (CH)
3	53.5 (CH)	54.6 (CH)	53.0 (CH)	52.9 (CH)	53.0 (CH)	52.9 (CH)	53.3 (CH)
4	96.2 (C)	95.1 (C)	96.3 (C)	96.5 (C)	96.1 (C)	96.3 (C)	97.0 (C)
5	115.9 (C)	118.3 (C)	115.0 (C)	114.7 (C)	115.3 (C)	114.4 (C)	114.9 (C)
6	144.5 (C)	144.4 (C)	143.7 (C)	143.7 (C)	143.6 (C)	143.4 (C)	144.2 (C)
7	117.8 (CH)	127.3 (CH)	117.6 (CH)	117.5 (CH)	117.5 (CH)	117.4 (CH)	117.9 (CH)
8	120.8 (CH)	121.3 (CH)	120.8 (CH)	120.7 (CH)	120.2 (CH)	120.7 (CH)	121.2 (CH)
9	152.4 (C)	156.4 (C)	152.0 (C)	152.4 (C)	151.7 (C)	152.9 (C)	152.8 (C)
10	116.8 (C)	110.8 (C)	117.4 (C)	117.5 (C)	116.9 (C)	117.2 (C)	117.6 (C)
1′	65.7 (CH)	65.9 (CH)	194.7 (C)	194.7 (C)	205.9 (C)	193.9 (C)	184.1 (C)
2′	36.8 (CH_2)	36.0 (CH_2)	43.3 (CH_2)	39.8 (CH_2)	72.4 (C)	41.3 (CH_2)	134.3 (CH)
3′	69.3 (CH)	70.0 (CH)	70.9 (CH)	38.7 (CH2)	40.2 (CH)	80.1 (CH)	143.1 (CH)
3′-OMe						59.4 (CH_3)	
4′	95.9 (C)	96.3 (C)	94.7 (C)	95.4 (C)	95.8 (C)	94.7 (C)	90.3 (C)
5′	117.7 (C)	117.1 (C)	121.5 (C)	121.7 (C)	115.0 (C)	121.5 (C)	122.2 (C)
6′	151.9 (C)	150.9 (C)	152.3 (C)	151.4 (C)	151.1 (C)	152.3 (C)	151.0 (C)
7′	115.2 (CH)	115.6 (CH)	122.0 (CH)	122.4 (CH)	115.5 (CH)	122.0 (CH)	121.8 (CH)
8′	131.6 (CH)	132.1 (CH)	131.7 (CH)	132.0 (CH)	131.9 (CH)	131.6 (CH)	132.4 (CH)
9′	121.6 (CH)	121.9 (CH)	120.4 (CH)	120.8 (CH)	119.5 (CH)	120.0 (CH)	121.2 (CH)
10′	142.4 (C)	142.6 (C)	132.0 (C)	131.8 (C)	144.0 (C)	131.9 (C)	131.8 (C)
11′					60.7 (CH_2)		
12′					37.2 (CH_2)		
13′					39.1 (CH_2)		

H-11′, as well as the HMBC correlations of H-3′ with C-5′, C-4′, and C-1′; H-13′ with C-12′, C-11′, and C-4′; H-12′ with C-10′, C-2′, C-3′, and C-4′; H-11′ with C-10′, C-2′, and C-1′; and *H*O-2′ with C-2′ and C-11′, permitted assignment of the structure of ymf 1029E as shown in **5**. The stereochemistry of ymf 1029E was deduced according to the observed HMBC correlation of βH-12′ at δ_H 2.50 ppm with C-10′ and NOESY correlation of βH-12′ with H-3′ and βH-11′, and αH-12′ with αH-11′, as shown in **5** (Figure 2), which was generated by MM2 calculation.[15]

Nematicidal activities of compounds **1**−**9** against the nematode *B. xylophilus* were determined. Our results indicated that all nine natural products displayed weak nematicidal activity, with IC_{50}'s between 100 and 200 μg/mL at the 24 h time point. Among the nine compounds, preussomerin D (**7**) was the most potent, while the two naphthalenone metabolites **8** and **9** had the weakest activity. The bis-spirobisnaphthalene pharmacophore appears to be critical for high activity because all tested bis-spirobisnaphthalene metabolites displayed more potent nematicidal activity than naphthalenone metabolites **8** and **9**. In comparison with commercial nematicide avermectin, active at a concentration of 0.25 μg/mL, these nine compounds identified here had much weaker nematocidal activities. However, they possess an unusual bis-spirobisnaphthalene/naphthalenone carbon skeleton that can be potentially exploited to make nematocides with greater efficiencies.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were taken on a Shimadzu double-beam 210A spectrophotometer. IR spectra were measured on a Perkin-Elmer-577 spectrophotometer. NMR spectra were recorded in CD_3COCD_3 solutions containing Me_4Si as an internal standard on Bruker AM-400 and DRX-500 spectrometers. The 1D and 2D NMR spectra were measured at 400 and 500 MHz, respectively. MS was performed on an Autospec-3000 spectrometer.

Cultivation of the Fungal Strain YMF1.01029. The producing fungus was isolated by L. Cai from the split of decaying branches of an unidentified tree near Lake Fuxian in Yunnan Province, China, and deposited in Yunnan Provincial Key Laboratory of Microbial Fermentation, Yunnan, China (culture collection number YMF1.01029). As the culture was nonsporulating, it could not be taxonomically characterized using spore characteristics. The culture was subcultured on PDA (potato 200 g, glucose 20 g, agar 18 g, and H_2O 1000 mL) agar at 25 °C for a period of 15 days. The mycelium-containing agar was then cut into pieces (1 × 1 cm) and inoculated into 30 × 250 mL Erlenmeyer flasks,

with each containing 50 mL of the seed media (PDB: potato 200 g, glucose 20 g, and H_2O 1000 mL) (10 pieces in each flask). After incubation at 25 °C for 15 days on a rotary shaker (190 rpm), each seed culture was transferred into a 500 mL Erlenmeyer flask containing 200 mL of liquid media (potato 200 g, glucose 20 g, peptone 2 g, yeast extract 4 g, and H_2O 1000 mL) and incubated at 25 °C for 10 days on a rotary shaker (180) rpm.

Isolation and Characterization of Compounds 1−9. A total of 60 L of cultural broth of the unidentified freshwater fungus YMF1.01029 was separated by filtration into the mycelia and filtrate. The filtrate was concentrated to 3000 mL and extracted three times with equal volumes of EtOAc. The EtOAc layer was dried over anhydrous $MgSO_4$ and evaporated to dryness under reduced pressure to obtain a brown gum (35.2 g), which showed antinematodal activities. This gum was loaded onto a silica gel column [1500 g silica gel G (200−300 mesh), 6.5 cm i.d. × 180 cm] and eluted with petroleum ether (bp 60−90 °C)/EtOAc/CH_3OH with increasing polarity to yield eight fractions (A−O) based on TLC behavior. Fraction D (735 mg), obtained on elution with 85% petroleum ether (bp 60−90 °C)/EtOAc, was further subjected to VLC [4 g silica gel G (400 mesh), 1.5 cm i.d. × 20 cm] using a gradient from 2% $CHCl_3$/EtOAc to 8% $CHCl_3$/EtOAc to give **9** (43 mg). Fraction F (1893 mg), obtained on elution with 30% petroleum ether (bp 60−90 °C)/EtOAc, was further separated on a Sephadex LH-20 gel column eluting with CH_3COCH_3 to afford a mixture of ymf 1029B and ymf 1029E (**2** and **5**, 33 mg), preussomerin D (**7**, 103 mg), and ymf 1029D (**4**, 31 mg). The mixture of ymf 1029B and ymf 1029E was purified further by reversed-phase semipreparative HPLC (Eclipse XDB-C-18 column; 5 μm; 9.4 × 250 mm, 2 mL/min, 60% CH_3OH/H_2O) to afford 3 mg of ymf 1029B and 10 mg of ymf 1029E. Fraction G (1562 mg), obtained on elution with 35% petroleum ether (bp 60−90 °C)/EtOAc, was further separated on a Sephadex LH-20 gel column eluting with CH_3COCH_3 to afford a mixture of ymf 1029C and preussomerin C (**3** and **6**, 43 mg) and ymf 1029A (**1**, 8 mg). The mixture of ymf 1029C and preussomerin C was rechromatographed by reversed-phase semipreparative HPLC using 65% CH_3OH/H_2O to afford 4 mg of ymf 1029C and 8 mg of preussomerin C. Fraction I (30 mg), obtained on elution with 45% petroleum ether (bp 60−90 °C)/EtOAc, was further separated on a Sephadex LH-20 gel column eluting with CH_3COCH_3 to yield pure **8** (12 mg).

Ymf 1029 A (1): orange, amorphous powder (CH_3COCH_3); $[\alpha]_D$ −260.8 (*c* 0.45, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 306.2 (3.23), 300.6 (3.25), 282.4 (3.22), 204.6 (4.32) nm; IR (film) ν_{max} 3417, 2926, 2857, 1697, 1611, 1594, 1484, 1433, 1352, 1332, 1280, 1160, 1120, 1089, 1069, 1049, 1024, 1002, 950, 904, 881, 820, 795, 751, 740, 701, 659, 626, 595, 552, 530, 510 cm^{-1}; EIMS *m/z* (rel int) 384 $[M]^+$ (100), 366 $[M - H_2O]^+$ (13), 322 (13), 320 (23), 304 (15), 291 (28), 267 (24), 265 (45), 263 (48), 249 (15), 237 (19), 221 (7), 209 (5), 192 (14), 175(28), 163(39), 149 (25), 147 (36), 121 (10); HRMS (ESITOF) *m/z* 407.0738 $[M + Na]^+$ (calcd for $C_{20}H_{16}O_8Na$, 407.0742).

Ymf 1029 B (2): orange, amorphous powder (CH_3COCH_3); $[\alpha]_D$ −298.8 (*c* 0.37,CH_3OH); UV (CH_3OH) λ_{max} (log ε) 308.2 (3.31), 303.6 (3.38), 286.3 (3.40), 209.6 (4.52) nm; IR (film) ν_{max} 3417, 3020, 2651, 1687, 1601, 1514, 1484, 1433, 1372, 1280, 1089, 1046, 1004, 990, 944, 881, 820, 797, 761, 743, 705, 639,625, 597, 552, 535, 510 cm^{-1}; EIMS *m/z* (rel int) 382 $[M]^+$ (100), 364 $[M - H_2O]^+$ (13), 320 (13), 310 (13), 302 (19), 289 (33), 265 (55), 263 (68), 249 (35), 237 (29), 221 (10), 209 (5), 192 (16), 175 (28), 163 (39), 149 (25); HRMS (ESITOF) *m/z* 405.0585 $[M + Na]^+$ (calcd for $C_{20}H_{14}O_8Na$, 405.0586).

Ymf 1029 C (3): pale yellow, amorphous solid (CH_3COCH_3); $[\alpha]_D$ −317.1 (*c* 0.51, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 306.2 (3.41), 303.6 (3.41), 255.6 (3.62), 202.4 (4.17) nm; IR (film) ν_{max} 3417, 2967, 2921, 1691, 1626, 1595, 1484, 1399, 1335, 1279, 1160, 1122, 1093, 1079, 1065, 1040, 1011, 991, 947, 929, 901, 879, 817, 797, 778, 752, 734, 697, 638, 594, 548, 505 cm^{-1}; EIMS *m/z* (rel int) 382 $[M]^+$ (100), 364 $[M - H_2O]^+$ (24), 336 (50), 335 (70), 318 (15), 307 (17), 294 (10), 293 (23), 263 (57), 236 (20), 191 (17), 175 (34), 161 (39), 149 (25), 134 (13), 91 (13); HRMS (ESITOF) *m/z* 405.0590 $[M + Na]^+$ (calcd for $C_{20}H_{14}O_8Na$, 405.0586).

Ymf 1029 E (4): orange, amorphous powder (CH_3COCH_3); $[\alpha]_D$ −308.1 (*c* 0.42, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 306.2 (3.35), 255.6 (3.56), 218.6 (4.02), 202.4 (4.12) nm; IR (film) ν_{max} 3423, 2924, 2854, 1691, 1593, 1484, 1414, 1357, 1335, 1283, 1248, 1203, 1159, 1123, 1079, 1047, 1006, 971, 943, 929, 902, 880, 846, 820, 807,793, 751, 719, 697, 593, 548, 501 cm^{-1}; EIMS *m/z* (rel int) 366 $[M]^+$ (100), 348 $[M - 18]^+$ (1), 362 (10), 294 (14), 265 (13), 251 (31), 213 (30), 211 (18), 209 (10), 202 (18), 193 (15), 167 (15), 129 (11); HRMS (ESITOF) *m/z* 389.0653 $[M + Na]^+$ (calcd for $C_{20}H_{14}O_7Na$, 389.0648).

Ymf 1029 E (5): pale yellow, amorphous solid (CH_3COCH_3); $[\alpha]_D$ −327.7 (*c* 0.36, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 303.6 (3.38), 255.6 (3.55), 202.2 (4.26) nm; IR (film) ν_{max} 3414, 2937, 2881, 1691, 1616, 1595, 1484, 1409, 1335, 1279, 1160, 1122, 1093, 1069, 1063, 1047, 1011, 993, 949, 909, 879, 827, 797, 768, 742, 704 cm^{-1}; (−)-FABMS *m/z* (rel int) 551 $[M + 1]^+$ (40), 404 $[M - H_2O]^+$ (42), 382 $[M - 2H_2O]^+$ (83), 357 (30), 347 (22), 335 (46), 329 (47), 307 (33), 291 (20), 263 (45), 243 (14), 189 (20), 173 (70), 161 (55), 145 (20), 133 (13), 91 (13); HRMS (ESITOF) *m/z* 445.0902 $[M + Na]^+$ (calcd for $C_{23}H_{18}O_8Na$, 445.0899).

Preussomerin C (6): orange, amorphous powder (CH_3COCH_3); $[\alpha]_D$ −157.8 (*c* 0.25, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 303.2 (3.61), 254.6 (2.70) nm; IR (film) ν_{max} 3695, 3648, 3357, 1020, 2857, 2684, 1589, 1517, 1469, 1414, 1328, 1277, 1209 cm^{-1}; EIMS *m/z* (rel int) 396 $[M]^+$ (100), 380 (14), 364 (15), 350(33), 335 (26), 320 (32), 292 (72), 263 (70), 236 (35), 221 (12), 205 (25), 190 (100), 173 (46), 161 (55), 147 (45), 133 (36), 119 (43), 105 (27), 91 (32), 77(43).

Preussomerin D (7): orange, amorphous powder (CH_3COCH_3); $[\alpha]_D$ −158.8 (*c* 0.35, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 296.2 (4.01), 254.6 (3.11) nm; IR (film) ν_{max} 3317, 1701, 1697, 1678, 1630, 1599, 1590, 1484, 1473, 1260 cm^{-1}; EIMS *m/z* (rel int) 364 $[M]^+$ (96), 346 (100), 318 (82), 289 (35), 261 (40), 161 (77), 149 (80), 77 (17).

4,6,8-Trihydroxy-3,4-dihydronaphthalen-1(2*H*)-one (6-hydroxy-isosclerone) (8): yellow powder (CH_3OH); $[\alpha]_D$ −53.78 (*c* 0.44, CH_3OH); EIMS *m/z* (rel int) 194 $[M]^+$ (100), 176 (4), 167 (15), 166 (38), 149 (46), 138 (55), 137 (100), 69 (6); ^1H NMR (CD_3OD, 400 MHz) δ_H 12.87 (1H, s, O*H*-8), 6.54 (1H, s, H-5), 6.17 (1H, s, H-7), 4.73 (1H, w, H-4, J = 10.1 Hz), 2.80 (1H, dt, H-2β, J = 18.2, 9.9, 4.8 Hz), 2.63 (1H, m H-2α), 2.26 (1H, m, H-3β), 2.04 (1H, m H-3α); ^{13}C NMR (CD_3OD, 400 MHz) δ_C 203.7 (C-1), 166.8 (C-6), 166.7 (C-8), 151.0 (C-4a), 110.2 (C-1a), 107.4 (C-5), 102.5 (C-7), 68.5 (C-4), 32.7 (C-3), 35.7 (C-2).

(4*RS*)-4,8-Dihydroxy-3,4-dihydronaphthalen-1(2*H*)-one (9): colorless powder (CH_3OH); $[\alpha]_D$ ±0 (*c* 0.44, CH_3OH); FABMS *m/z* (rel int) 363 $[M + 2$ glycerol $+ 1]^+$ (75), 271 $[M +$ glycerol$+1]^+$ (15), 179 $[M + 1]^+$ (100); ^1H NMR (CD_3COCD_3, 500 MHz) δ_H 12.33 (1H, s, OH-8), 7.42 (1H, t, J = 7.95 Hz, H-6), 7.00 (1H, d, J = 7.45 Hz, H-5), 6.71 (1H, d, J = 8.20 Hz, H-7), 4.78 and 4.61 (1H, m, H-4), 2.78 (1H, dt, H-2β, J = 18.2, 9.9, 4.8 Hz), 2.62 (1H, m, H-2α), 2.20 (1H, m, H-3β), 2.00 (1H, m, H-3α); ^{13}C NMR (CD_3COCD_3, 500 MHz) δ_C 206.0 (C-1), 163.7 (C-8), 149.5 (C-4a), 137.9 (C-6), 118.7 (C-5), 117.5 (C-7), 116.0 (C-1a), 68.2 (C-4), 36.3 (C-2), 33.0 (C-3).

Culture and Preparation of Nematodes. The test nematodes *B. xylophilus* were cultured and prepared as described in the literatures.[16]

Assay of the Nematicidal Activity. Each test compound was dissolved in DMSO and diluted to 200 and 100 μg/mL by 0.5% emulsified H_2O (5 mL of Tween 80 in 1 L of distilled H_2O) for the nematicidal activity assay. As a standard, avermectin was used and 3% DMSO and 0.5% emulsified H_2O were also used as control. The numbers of active and inactive nematodes were counted at 24 and 48 h, respectively. Nematodes were considered to be dead if they showed no response to physical stimuli, and the toxicity was estimated on the basis of the percentage of dead nematodes. Each treatment was replicated three times, and the mean percentage mortality was calculated. The experiments were repeated twice.

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Supporting Information Available: Nematicidal activity against the *Bursaphelenchus xylophilus* nematode. This material is available free of charge via the Internet at http://pubs.acs.org.

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