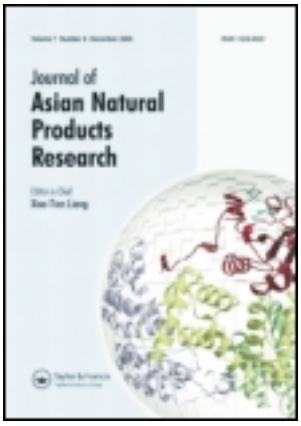

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Three new compounds from soil actinomycete *Streptomyces albospinus* 15-4-2

Lei Yu[a,b†], Hao-Fu Dai[a,b†], You-Xing Zhao[a,b], Yan-Bo Zeng[a,b], Wen-Ting Jiang[a,b], Wen-Li Mei[a,b]* and Hui-Cai Zeng[a,b]*.

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Three new compounds, 2-methyl-2,5,6-bornantriol (**1**), 4,4′-(3-hydroxypropane-1,1-diyl)diphenol (**2**), and 7-(4-methoxybenzyl)-4,5,6,7-tetrahydro-1,3-oxazepine-5,6-diol (**3**), were isolated from the fermentation broth of the soil actinomycete *Streptomyces albospinus* 15-4-2. Their structures were completely elucidated using the combination of 1D, 2D NMR techniques (COSY, HMQC, HMBC, and ROESY), and HR-ESI-MS analysis. None of the compounds **1**–**3** showed any inhibitory effect on *Fusarium oxysporum* f.sp. *cubense* race 4.

Keywords: soil actinomycete; *Streptomyces albospinus*; chemical constituents

1. Introduction

Filamentous soil bacteria belonging to the genus *Streptomyces* are Gram-positive bacteria, characterized by a complex morphologic differentiation cycle accompanied by the production of numerous extracellular enzymes, as well as many kinds of bioactive secondary metabolites having great structural and functional diversity [1,2]. They are widely distributed in a variety of natural and man-made environments, constituting a significant component of the microbial population in most soils [3,4]. As a group, the *Streptomycetes* provide nearly 80% of all the world's antibiotics [5], so they constitute a leading source of novel molecules for the development of new drug candidates [6]. In our screening for an antimicrobial agent from actinomycetes isolated from soil sample collected in Bawangling tropical virgin forest to deal with *Fusarium oxysporum* f.sp. *cubense* race 4, the pathogenic fungus caused banana wilt disease, the fermentation broth of the soil actinomycete *Streptomyces albospinus* 15-4-2 showed antimicrobial activity against *F. oxysporum* f.sp. *cubense* race 4. In this paper, we describe the fermentation, isolation, and structural elucidation of new compounds **1**–**3** from the fermentation broth of the soil actinomycete *S. albospinus* 15-4-2 (Figure 1).

2. Results and discussion

Compound **1** was obtained as a colorless oil and had a molecular formula $C_{11}H_{20}O_3$ based on its HR-ESI-MS at *m/z* 235.1103 $[M + Cl]^-$, which was supported by ^{13}C NMR and DEPT spectral data (Table 1). The IR spectrum showed characteristic

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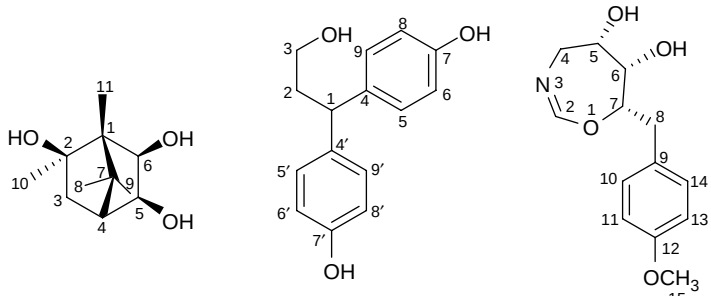

1 **2** **3**

Figure 1. The structures of compounds **1**–**3**.

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of compounds **1** and **3** (in CD_3OD, δ_{ppm}, J_{Hz}).

	1		**3**	
No.	δ_C	δ_H	δ_C	δ_H
1	59.9 (s)			
2	80.2 (d)		163.9 (d)	7.77 (1H, s)
3	38.3 (t)	1.80 (1H, m) 1.95 (1H, br d, $J = 12.9$)		
4	52.4 (d)	1.80 (1H, m)	50.7 (t)	3.38 (1H, dd, $J = 12.8, 2.4$) 3.62 (1H, dd, $J = 12.8, 5.5$)
5	81.2 (d)	4.19 (1H, dd, $J = 2.1, 1.9$)	74.2 (d)	4.12 (1H, m)
6	82.9 (d)	3.56 (1H, d, $J = 2.1$)	77.1 (d)	3.91 (1H, t, $J = 4.4$)
7	51.1 (s)		63.3 (d)	4.05 (1H, m)
8	24.8 (q)	1.10 (3H, s)	35.0 (t)	2.82 (1H, dd, $J = 13.5, 7.9$) 3.03 (1H, dd, $J = 13.5, 7.0$)
9	23.2 (q)	1.03 (3H, s)	131.1 (s)	
10	26.7 (q)	1.25 (3H, s)	131.6 (d)	7.17 (1H, d, $J = 8.6$)
11	6.8 (q)	0.84 (3H, s)	115.1 (d)	6.85 (1H, d, $J = 8.6$)
12			160.0 (s)	
13			115.1 (d)	6.85 (1H, d, $J = 8.6$)
14			131.6 (d)	7.17 (1H, d, $J = 8.6$)
15			55.7 (q)	3.75 (3H, s)

absorption for hydroxyl groups (3672 cm^{-1}). The ^{13}C NMR and DEPT spectra showed 11 carbon resonances, including four methyls, one methylene, three methines (two oxygenated), and three quaternary carbons (one oxygenated), which indicated a monoterpene derivative. The ^{13}C NMR spectral data of compound **1** were similar to a known compound, 2-methyl-2,5-bornandiol [7], except for the appearance of an additional oxygenated methine group at δ_C 82.9 in **1** and the absence of a methylene group (C-6) in the 2-methyl-2,5-bornandiol, which suggested that C-6 of compound **1** was substituted by a hydroxyl group consistent with its molecular formula. The ^{1}H–^{1}H COSY correlations from H-5 (δ 4.19) to H-4 (δ 1.80) and H-6 (δ 3.56) further supported that the hydroxyl group was linked to C-6, which was confirmed by the HMBC correlation from H-11 (δ 0.84) to

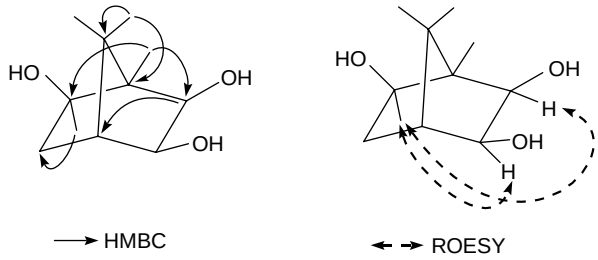

Figure 2. Key HMBC and ROESY correlations for compound **1**.

C-6 (δ 82.9) (Figure 2). The relative stereochemistry of **1** was similar to 2-methyl-2,5-bornandiol exhibiting α-orientations of CH_3-10 and H-5, which was confirmed by the ROESY correlation of H-5 (δ 4.19) with H-10 (δ 1.25) (Figure 2). The β-orientation of 6-OH was determined on the basis of ROESY correlation of H-6 (δ 3.56) with H-10 (δ 1.25). Based on the above evidence, compound **1** was elucidated to be 2-methyl-2,5,6-bornantriol as shown in Figure 1.

Compound **2** was isolated as a colorless oil and its molecular formula was determined to be $C_{15}H_{16}O_3$ based on its HR-ESI-MS at *m/z* 243.1026 $[M - H]^-$, which was supported by ^{13}C NMR and DEPT spectral data. The IR spectrum showed characteristic absorption for hydroxyl groups (3567 cm^{-1}) and phenyl moiety (1456 cm^{-1}, 1495 cm^{-1}, 1596 cm^{-1}). The ^{13}C NMR and DEPT spectra (Table 2) showed 15 carbon resonances, including two methylene (one oxygenated), nine methine (six overlapped), and four quaternary (two oxygenated and two overlapped) carbons. The ^1H NMR spectrum of compound **2** showed aromatic proton signals at δ 7.04 (4H, d, $J = 8.4$ Hz) and δ 6.68 (4H, d, $J = 8.4$ Hz). The ^{13}C NMR spectrum of **2** showed 12 aromatic carbon signals at δ 137.8 × 2, 129.7 × 4, 156.5 × 2 and 116.1 × 4. Hence, compound **2** was considered to contain two *para*-substituted benzene moieties. The ^{13}C NMR spectral data of **2** were similar to those of a known compound, 4,4′-(4-hydroxybutane-1,1-diyl) diphenol [8], except for the lack of the methylene at δ 32.2 in 4,4′-(4-hydroxybutane-1,1-diyl) diphenol. Therefore, the structure of **2** was established as 4,4′-(3-hydroxypropane-1,1-diyl) diphenol.

Compound **3** was obtained as a colorless oil and gave a molecular formula of $C_{13}H_{17}NO_4$ with six degrees of unsaturation, as deduced by its HR-ESI-MS at *m/z* 252.1232 $[M + H]^+$, which was supported by ^{13}C NMR and DEPT spectral data (Table 1). The IR spectrum showed

Table 2. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of compound **2** (in CD_3OD, δ_{ppm}, J_{Hz}).

No.	δ_C	δ_H	No.	δ_C	δ_H
1	46.9 (d)	3.91 (1H, t, $J = 8.0$)	9	129.7 (d)	7.04 (1H, d, $J = 8.4$)
2	39.9 (t)	2.17 (2H, dt, $J = 8.0$, 6.8)	4′	137.8 (s)	
3	61.4 (t)	3.45 (2H, t, $J = 6.8$)	5′	129.7 (d)	7.04 (1H, d, $J = 8.4$)
4	137.8 (s)		6′	116.1 (d)	6.68 (1H, d, $J = 8.4$)
5	129.7 (d)	7.04 (1H, d, $J = 8.4$)	7′	156.5 (s)	
6	116.1 (d)	6.68 (1H, d, $J = 8.4$)	8′	116.1 (d)	6.68 (1H, d, $J = 8.4$)
7	156.5 (s)		9′	129.7 (d)	7.04 (1H, d, $J = 8.4$)
8	116.1 (d)	6.68 (1H, d, $J = 8.4$)			

characteristic absorption for hydroxyl groups (3594 cm^{-1}) and phenyl moiety (1460 cm^{-1}, 1497 cm^{-1}, 1601 cm^{-1}). The ^{13}C NMR and DEPT spectra (Table 1) showed 13 carbon resonances, including one methoxyl, two methylene, eight methine (four oxygenated), and two quaternary carbons. The ^{1}H NMR spectrum of compound **3** showed aromatic proton signals at δ 7.17 (2H, d, $J = 8.6$ Hz) and δ 6.85 (2H, d, $J = 8.6$ Hz) characterized for an AA′BB′ spin system, while the ^{13}C NMR spectrum showed corresponding signals at δ 131.1 × 2 and 115.1 × 2. Moreover, the ^{1}H NMR spectrum showed a methoxy signal at d 3.75 (3H, s), while ^{13}C NMR spectrum showed corresponding signal at δ 55.7. The methoxyl was connected with C-12 on the basis of HMBC correlation of the signal at δ 3.75 (H-OCH_3) with the carbon at δ 160.0 (C-12). In the $^{1}H-^{1}H$ COSY spectrum, the correlations from H-7 (δ 4.05) to H-6 (δ 3.91) and H-8 (δ 2.82, 3.03), H-5 (δ 4.12) to H-4 (δ 3.38, 3.62) and H-6 (δ 3.91) determined the connections of chain C-4–C-8. The HMBC correlation from H-8 (δ 2.82, 3.03) to C-10 and C-14 (δ 131.6) indicated that C-8 was linked to C-9. In the ^{13}C NMR spectrum, the characteristic chemical shift of C-4 (δ 50.7) indicated that C-4 was linked to a nitrogen atom, and the downfield shift of olefinic carbon signal C-2 (δ 163.9) indicated that C-2 was linked to an oxygen atom. The key correlations from H-2 (δ 7.77) to C-4 (δ 50.7) and C-7 (δ 63.3) in the HMBC spectrum confirmed that C-2 was connected with C-4 and C-7 via a nitrogen atom and an oxygen atom, respectively, forming a seven-membered ring contained one olefinic bond between C-2 and the nitrogen atom. The relative configurations of the chiral carbons (C-5, C-6, C-7) of **3** were proposed by a ROESY experiment exhibiting correlations of H-4β/H-6, 7 and H-5/H-7, suggesting that H-5, H-6, and H-7 were on the same side (Figure 3). Based on the above evidence, compound **3** was determined as 7-(4-methoxybenzyl)-4,5,6,7-tetrahydro-1,3-oxazepine-5,6-diol.

None of the compounds **1**–**3** showed any inhibitory effect on *F. oxysporum* f.sp. *cubense* race 4. Further chemical research is going on to find out the antimicrobial component that contributed to the antimicrobial activity of the fermentation broth of the soil actinomycete *S. albospinus* 15-4-2.

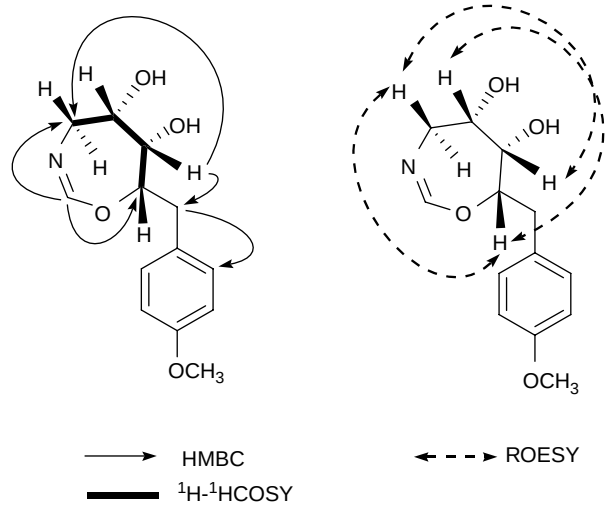

Figure 3. Key $^{1}H-^{1}H$ COSY, HMBC, and ROESY correlations for compound **3**.

3. Experimental

3.1 General experimental procedures

Optical rotation was recorded using a Rudolph Autopol III polarimeter (Rudolph Research Analytical, NJ, USA). The UV spectrum was measured on a Shimadzu UV-2550 spectrometer. The IR spectrum was obtained on a Nicolet 380 FT-IR instrument from KBr pellets. The NMR spectra were recorded on a Bruker AV-400 spectrometer, using TMS as an internal standard. The HR-ESI-MS was measured with an API QSTAR Pulsar mass spectrometer. Column chromatography (CC) was performed with silica gel (Marine Chemical Industry Factory, Qingdao, China) and Sephadex LH-20 (Merck, Darmstadt, Germany). TLC was performed with silica gel GF254 (Marine Chemical Industry Factory).

3.2 Actinomycete material

The producing organism, *S. albospinus* 15-4-2, was isolated from a soil sample collected in Bawangling tropical virgin forest, Hainan, China. This actinomycete was provided by Prof. Hui-Cai Zeng, from the Institute of Tropical Bioscience and Biotechnology, Haikou, maintained on potato dextrose agar slants containing 0.2% $CaCO_3$ and 0.2% $MgSO_4$ at 4°C, which was identified as *S. albospinus* by the analysis of the microscopical morphological characters and 16 s rDNA sequence.

3.3 Fermentation, extraction, and isolation

The producing strain, *S. albospinus* 15-4-2, was grown on a solid medium composed of potato (200 g/l), glucose (20 g/l), agar (20 g/l), $CaCO_3$ (0.2 g/l), and $MgSO_4{\cdot}5H_2O$ (0.2 g/l) at room temperature for 72 h. Three pieces of mycelial agar plugs ($0.5 \times 0.5\,cm^2$) were inoculated into 1l Erlenmeyer flasks containing 300 ml of liquid medium composed of potato (20 g/l), glucose (10 g/l), $CaCO_3$ 0.2 g/l), and $MgSO_4$ (0.2 g/l). The mixture was shaken at 160 rpm at room temperature for 7 days, and then it was kept still at room temperature for 21 days. The culture broth (120 l) was filtered through cheesecloth to separate into filtrate and mycelia. The filtrate was concentrated under reduced pressure to approximately a quarter of the original volume and then partitioned with petroleum ether, ethyl acetate, and *n*-butanol, successively. The combined ethyl acetate extract was concentrated under reduced pressure to give a crude extract (18.6 g). The crude extract was separated into 16 fractions on a flash silica gel column using a step gradient elution of $CHCl_3$–CH_3OH (1:0-0:1, v/v). Fraction 11 (1.1 g) was subjected to CC over Sephadex LH-20 eluted with $CHCl_3$–CH_3OH (1:1, v/v), then submitted to repeated CC on silica gel eluting with petroleum ether–acetone (2:1, v/v) and $CHCl_3$–MeOH (20:1, v/v), and finally yielded compounds **1** (3.8 mg) and **2** (6.9 mg). Fraction 9 (1.0 g) was purified by CC using Sephadex LH-20 ($CHCl_3$–CH_3OH 1:1, v/v), then silica gel CC eluting with petroleum ether–acetone (3:1, v/v) to yield **3** (7.9 mg).

3.3.1 2-Methyl-2,5,6-bornantriol (*1*)

A colorless oil; $[\alpha]_D^{26}$ −6.0 ($c = 0.85$, MeOH); IR ν_{Max}^{KBr} (cm^{-1}): 3672, 2954; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data see Table 1; HR-ESI-MS: *m/z* 235.1103 $[M + Cl]^-$ (calcd for $C_{11}H_{20}O_3Cl$, 235.1100).

3.3.2 4,4′-(3-Hydroxypropane-1,1-diyl) diphenol (*2*)

A colorless oil; $[\alpha]_D^{26}$ +19.0 ($c = 0.82$, MeOH); UV (MeOH): λ_{max} (log ε) 236 (3.70), 217 (3.41) nm; IR ν_{Max}^{KBr} (cm^{-1}): 3567, 1456, 1495, 1596; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data see Table 2; HR-ESI-MS: *m/z* 243.1026 $[M - H]^-$ (calcd for $C_{15}H_{15}O_3$, 243.1021).

*3.3.3 7-(4-Methoxybenzyl)-4,5,6,7-tetrahydro-1,3-oxazepine-5,6-diol (**3**)*

A colorless oil; $[\alpha]_D^{26}$ −20.5 ($c = 0.79$, MeOH); UV (MeOH): λ_{max} (log ε) 193 (5.00), 276 (1.43), 220 (3.40) nm; IR ν_{Max}^{KBr} (cm^{-1}): 3594, 2962, 1460, 1497, 1601; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data see Table 1; HR-ESI-MS: *m/z* 252.1232 $[M + H]^+$ (calcd for $C_{13}H_{18}NO_4$, 252.1235).

3.4 Antimicrobial activity

All the three compounds were tested for *in vitro* antimicrobial activity against *F. oxysporum* f.sp. *cubense* race 4 (obtained from Prof. Hui-Cai Zeng of the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences) by the filter paper disc agar diffusion method [9]. None of the three compounds showed any inhibitory effect against *F. oxysporum* f.sp. *cubense* race 4.

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