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ScienceDirect
Phytochemistry 69 (2008) 1439–1445
PHYTOCHEMISTRY
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Stereumin A–E, sesquiterpenoids from the fungus *Stereum* sp. CCTCC AF 207024

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Received 22 October 2006; received in revised form 19 January 2008
Available online 5 March 2008

Abstract

Five cadinane sesquiterpenoids, named stereumin A (**1**), B (**2**), C (**3**), D (**4**) and E (**5**) were isolated from the $CHCl_3$ extract of the culture broth of the fungal strain CCTCC AF 207024. Based on the sequences at the internal transcribed spacer (ITS) region and partial 28S rDNA, this fungus was identified as a *Stereum* sp. The structures of the five compounds were elucidated using spectroscopic data from 1D, 2D NMR and HRESIMS experiments, and the structures of **1** and **2** were further confirmed by single-crystal X-ray diffraction analysis. Compounds **1**–**5** showed nematicidal activities against the nematode *Panagrellus redivivus* at 400 mg l^{-1}. Among these five compounds, compounds **3** and **4** killed 84.4% and 94.9% of *P. redivivus*, respectively in 48 h.

Keywords: *Stereum* sp. CCTCC AF 207024; ITS and 28S rDNA sequences; Sesquiterpenoid; Nematicidal

1. Introduction

Microbial metabolites have been used extensively in agriculture and medicine (Lange, 1996; Pelaez, 2006). In the investigation of the chemical constituents of strains in the fungal genus *Stereum*, a series of new sesquiterpenoids have been reported. These include sesquiterpenoids of the hirsutane type (Mellows et al., 1973; Yun et al., 2002; Yoo et al., 2006), the sterpurane type (Ayer et al., 1981; Xie et al., 1992) and the cadinane type (Li et al., 2006). Among these compounds, several were found to possess antibacterial activities (Yun et al., 2002) as well as scavenger activities against superoxide anion radicals (Yoo et al., 2006).

The crude $CHCl_3$ extract from culture broth of *Stereum* sp. CCTCC AF 207024 showed nematicidal activity against the nematode *Panagrellus redivivus* (Linn.) Goodey. Bioassay-guided fractionations of the extract resulted in five novel cadinane sesquiterpenoids stereumin A–E (**1**–**5**). Below we discuss the fungal strain and its five nematicidal sesquiterpenoids.

2. Results and discussion

2.1. Identification of strain CCTCC AF 207024

A total of 556 nucleotides from the internal transcribed spacer (ITS) region and 903 from the partial 28S rDNA were obtained from strain CCTCC AF 207024 (GenBank accession number EF067346 and EF600046, respectively). Similar sequences were obtained with a BLAST search from GenBank. Among 12 sequences most similar to EF067346, 6 sequences (AF533962, AY618670, AY781272, AY854063, AY805632 and DQ000294) were obtained from strains belonging to *Stereum*, which shared a high degree of similar-

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doi:10.1016/j.phytochem.2008.01.012

ity (92–95%) with EF067346, and six other similar sequences were from an unidentified basidiomycete. Likewise, of the 12 sequences most similar to EF600046, 10 sequences were obtained from strains of *Stereum* (sharing 98–99% similarity with EF600046), and only AF042563 was gained from an unidentified basidiomycete and AY293189 from *Hypsizygus*. Based on the above results, strain CCTCC AF 207024 is identified as a member of the genus *Stereum*.

2.2. Identification structures of compounds 1–5

The $CHCl_3$ extract of fermentation broth of *Stereum* sp. CCTCC AF 207024 was purified by Silica Gel and Sephadex LH-20 column chromatographic steps to afford compounds **1**–**5** (Fig. 1).

Stereumin A (**1**) was obtained as colorless needles (acetone). The HRESIMS data determined the molecular formula to be $C_{15}H_{22}O_4$ ($[M+Na]^+$ at *m/z* 289.1424). The DEPT experiments (Table 1) showed 15 C signals for two methyl, four methylenes, five methines, and four quaternary C-atoms including one terminal C=C bond (δ 110.9 and 148.0) and one C=C bond (δ 126.1 and 135.6). The presence of the cadinane sesquiterpenoid skeleton was deduced from the 2D NMR spectra including analyses of HMQC, HMBC and ^{1}H,^{1}H-COSY spectra (Bohlmann et al., 1982). The HMBC data (Table 1) showed ^{1}H,^{13}C NMR long-range correlations between: the protons of the terminal C=C bond methylene at δ 5.57 and 5.14 (1-CH_2) and the C-atoms C-1, C-9b and C-2; one proton of the oxygenated methylene at δ 4.63 (H_α-2) and the C-atoms C-1, 1-CH_2, C-3a, and 4.27 (H_β-2) and the C-atom C-9b to afford the furan ring unit **1a** (Fig. 2). Meanwhile, the HMBC data (Table 1) showed correlations between: the methine proton at δ 6.05 (H-9) and C-7, C-9a, C-5a; the proton of the oxygenated methine at δ 4.04 (H-7) and C-8, C-6, C-5a; the proton at δ 2.19 (H-9a) and C-8, C-6, and the proton at δ 1.39 (H_β-4) and C-5, 5-CH_3, together with the ^{1}H,^{1}H-COSY connection between the 5-CH_3 and H-5, which provided the fragment **1b** (Fig. 2). Fragments **1a** and **1b** were connected according to the correlations between the H-9a and C-9b, H_β-4 and C-3a. The relative configuration was deduced from ROESY experiments based on correlations between H-9a and H-5, and between H-5a and 5-CH_3 (Fig. 2). The final proof of the structure and stereochemistry of compound **1** was obtained from a single crystal X-ray analysis. The ORTEP diagram of the crystal structure of this compound (Fig. 3) clearly shows that the protons 5-CH_3, 5a-H and 7-OH have α-orientations. It also shows that 3a-OH, 9a-H and 9b-OH have β-orientations, respectively. Compound **1** is then elucidated as (3a*R**,5*S**,5a*R**,7*S**,9a*R**,9b*S**)-5,8-dimethyl-1-

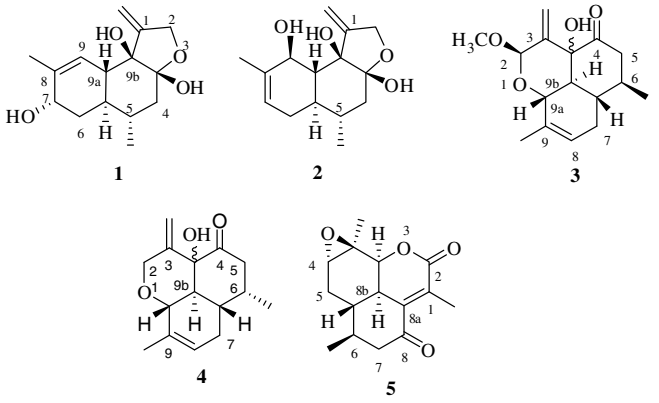

Fig. 1. Structures of compounds **1**–**5** and their relative configurations.

Table 1
^{1}H, ^{13}C and HMBC spectroscopic data for stereumin A (**1**) and B (**2**)

Position	Stereumin A (**1**)			Stereumin B (**2**)		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1	\	148.0	\	\	148.3	\
2	4.63 *dd* (2.2, 12.0)	67.5	1, 1-CH_2, 3a	4.65 *d* (12.5)	68.3	1, 1-CH_2, 3a, 9b
	4.27 *dd* (1.5, 13.0)		1,1-CH_2, 9b	4.18 *d* (12.6)		1, 1-CH_2, 3a, 9b
3	\	106.2	\	\	105.0	\
4	1.93 *m*	42.6	9, 5a	1.87 *dd* (3.7, 10.2)	40.7	3a, 9b, 5, 5-CH_3
	1.39 *m*		3a, 5, 5-CH_3	1.31 *m*		3a, 6, 5-CH_3
5	1.50 *m*	33.9	4	1.50 *m*	35.7	5a, 6, 5-CH_3
5a	1.36 *dt* (3.5, 11.4)	36.5	4, 5-CH_3	1.37 *m*	39.8	9, 9a, 6, 5-CH_3
6	2.16 *br s*	36.6	8, 7, 9a, 5a	2.29 *br d*(17.4)	30.8	8, 7, 9a, 5a
	1.45 *m*		\	1.71 *m*		\
7	4.04 *br s*	68.0	8, 9, 6, 5a, 8-CH_3	5.54 *br s*	124.3	9, 5a, 6, 8-CH_3
8	\	135.6	\	\	135.9	\
9	6.05 *br s*	126.1	9b, 7, 9a, 5a, 8-CH_3	4.89 *d* (8.7)	71.5	8, 7, 9b, 9a
9a	2.19 *m*	47.5	8, 9b, 6	1.94 *dd* (3.2, 9.25)	49.6	1, 9b,9, 5a, 5, 6
9b	\	77.0	\	\	78.5	\
CH_2(1)	5.57 *d* (2.1)	110.9	1, 9b, 2	5.37 *s*	110.0	1, 9b, 2
	5.14 *d* (1.6)		1, 9b, 2(w)	5.15 *s*		1, 9b, 2
CH_3(5)	1.01 *d* (6.1)	18.5	3, 4, 6, 5, 5a	0.89 *s*	19.1	3a, 4, 5
CH_3(8)	1.91 *s*	20.7	8, 9, 7, 6	1.77 *s*	19.3	8, 7, 9

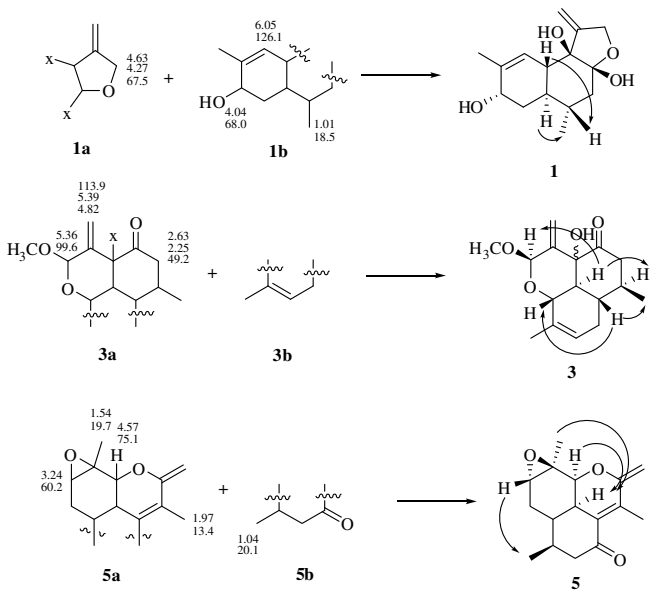

Fig. 2. Fragments of compounds **1**, **3** and **5** and their NOEs.

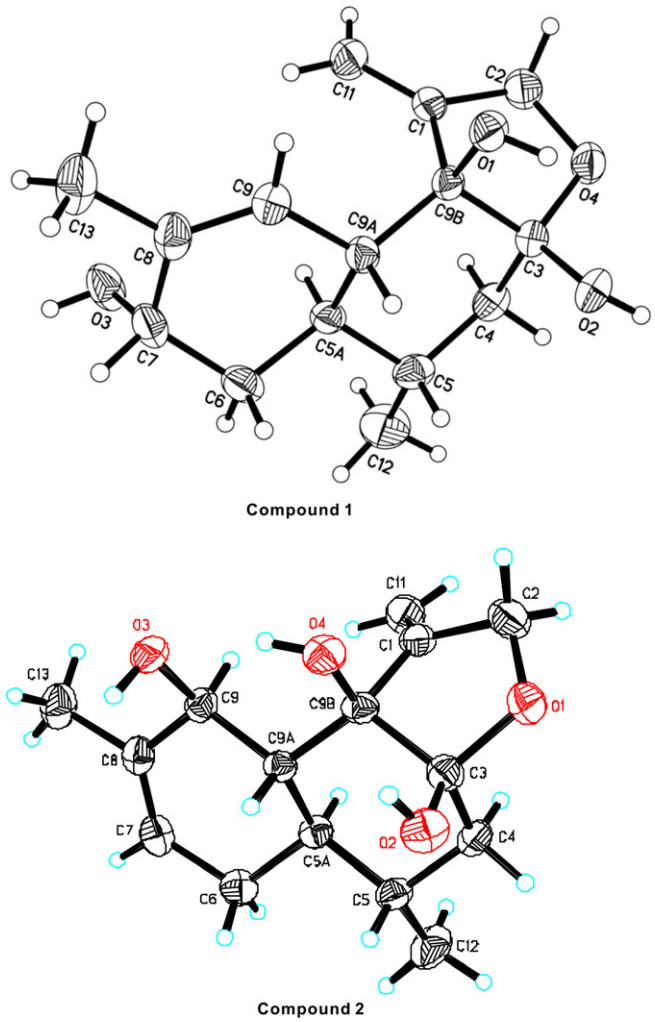

Fig. 3. ORTEP diagram of the crystal structures of compounds **1** and **2**.

methylene-1,2,3a,4,5,5a,6,7,9a,9b-decahydronaphtho[2,1-b]furan-3a,7,9b-triol.

Stereumin B (**2**) was obtained as colorless cuboid crystals (isopropyl alcohol). The HRESIMS data determined the molecular formula to be $C_{15}H_{22}O_4$ ([M–H]$^-$ at *m*/*z* 265.1439). The DEPT experiments (Table 1) showed 15 C signals including two C=C bonds ((δ 110.0 and 148.3) and (δ 124.3 and 135.9)). The IR and NMR spectroscopic data of compound **2** were very similar to those of compound **1**. After careful analysis the HMBC data (Table 1), the results showed that one double bond (δ 124.3 and 135.9) and one hydroxyl group in compound **2** were at different positions on the decaline ring unit compared to compound **1**. ^{1}H,^{1}H-COSY plots indicated that 5-CH_3 was correlated with H-5, and between H-9 and H-9a, H-7 and H_α-6, together with other data of HMBC (Table 1) that established the structure of **2**. The relative configuration was elucidated from ROESY experiments with correlations between H-9a and H-5, as well as between H-9 and H-5a. Final proof of the structure and stereochemistry of compound **2** was also obtained from a single crystal X-ray analysis. The ORTEP diagram of the crystal structure of this compound (Fig. 3) clearly shows that the protons 5-CH_3 and 5a-H have the α-orientations. It also shows that 3a-OH, 9-OH, 9a-H and 9b-OH have the β-orientations, respectively. Compound **2** is then elucidated as (3a*R**,5*S**,5a*R**,9*S**,9a*R**,9b*S**)-5,8-dimethyl-1-methylene-1,2,3a,4,5,5a,6,9,9a,9b-decahydronaphtho[2,1-b]furan-3a,9,9b-triol.

Stereumin C (**3**) was obtained as colorless powder. The HRESIMS data determined the molecular formula to be $C_{16}H_{22}O_4$ ([M+Na]$^+$ at *m*/*z* 301.1408). The ^{1}H and ^{13}C NMR spectra indicated 16 C signals including one terminal double bond (δ 113.9 and 145.8), one double bond (δ 121.1 and 137.1) and one ketone group (δ 211.7). The cadinane skeleton was deduced from the 2D NMR spectroscopic data (Table 2). The HMBC data showed ^{1}H,^{13}C NMR long-range correlations between the terminal methylene protons at δ 5.39 and 4.82 (3-CH_2) to C-3, C-2, C-3a, H-2 to C-3/C-9a, between H-9b and C-7, C-6a, C-9a, C-3a, C-9, C-3, C-4, and between H_α-5 and 6-CH_3, C-6, C-6a, C-3a, C-4. Together with the ^{1}H,^{1}H-COSY correlations between 6-CH_3 and H-6, H-9b and H-9a, H-6a, these data suggested the chromene unit **3a** (Fig. 2). Similarly, the ^{1}H,^{1}H-COSY correlation between H-8 and H_α-7, and the HMBC correlation between H_α-7 and C-8, C-9 indicated fragment **3b** (Fig. 2). The connectivity of fragments **3a** and **3b** was established by correlations between 9-CH_3 and C-9a, H-6a and C-7, H_α-7 and C-5 (w). The ROESY experiments established the relative configuration on the basis of correlations between H-9b and H-2, H-6, and between H-6a and H-9a, 6-CH_3 (Fig. 2). Compound **3** was thus elucidated to be (2*R**,3*S**,6*R**,6a*S**,9a*R**)-3,3a,5,6,6a,7-hexahydro-3a-hydroxy-2-methoxy-6,9-dimethyl-3-methylenebenzo[*de*]-chromen-4(2*H*,9a*H*)-one.

Stereumin D (**4**) was obtained as colorless powder. The HRESIMS data determined the molecular formula to be

Table 2
^1H, ^{13}C and HMBC spectroscopic data for stereumin C (**3**) and D (**4**)

Position	Stereumin C (**3**)			Stereumin D (**4**)		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
2	5.36 *d* (1.5)	99.5	3, 9a	4.49 *d* (12.7)	70.8	3-CH_2, 2
				4.19 *d* (12.8)		3-CH_2, 2
3	\	145.8	\	\	144.8	\
3a	\	77.0	\	\	78.7	\
4	\	211.7	\	\	211.8	\
5	2.63 *dd* (2.6, 14.2)	49.2	6-CH_3, 6, 6a, 3a, 4	2.61 *d* (10.1)	47.9	6, 3a, 5
	2.25 *t* (13.4)		6-CH_3, 6, 6a, 4	2.58 *m*		6, 3a, 5
6	1.75 *m*	35.5	\	1.75 *m*	35.1	\
6a	1.52 *m*	43.4	7, 6	1.58 *m*	40.1	7
7	2.34 *br d* (17.2)	32.3	5(w), 8, 9	2.43 *br d* (17.5)	32.1	6a, 8, 9
	1.89 *m*		8, 9	1.61 *m*		6a, 8, 9
8	5.31 *br s*	121.1	\	5.61 *br*	125.6	\
9	\	137.1	\	\	133.4	\
9a	4.13 *br d* (9.45)	73.9	\	4.08 *d* (2.0)	74.7	6, 8, 9
9b	2.12 *t* (11.0)	55.5	7, 6a, 9a, 3a, 9, 3, 4	1.79 *m*	52.2	\
3-CH_2	5.39 *d* (1.5)	113.9	3, 2, 3a	5.05 *s*	113.3	2, 3a, 3
	4.82 *d* (1.9)		3, 2, 3a	4.65 *s*		2, 3a, 3
6-CH_3	1.00 *d* (6.4)	18.6	6, 6a, 5, 4(w)	1.06 *d* (6.4)	19.4	6a, 6
9-CH_3	1.86 *s*	17.4	7, 9a, 8, 9	1.82 *s*	21.0	9a, 8, 9
2-OCH_3	3.50 *s*	55.3	2	\	\	\

$C_{15}H_{20}O_3$ ($[M+Na]^+$ at *m/z* 271.1318). The IR and NMR spectroscopic data of compound **4** were very similar to those of compound **3**. After analysis of the HMBC data (Table 2), the results showed one additional methoxy group was at position C-2 in compound **3**, so the chemical shift value of C-2 of compound **4** was at δ 70.8 instead of at δ 99.5 in compound **3**. The HMBC data (Table 2) and the ^1H,^1H-COSY correlations indicated that H-9a was correlated with H-9b, H-9b with H-6a, H-6a with H-6, and H-8 with H-7. Finally, ROESY experiments showed NOE correlations between H-9a and H-6a, H-9b and 6-CH_3, H-6 and H-9a to support its relative configuration. From the above data, the structure of **4** was determined to be (3*S**,6*S**,6a*S**,9a*R**)-3,3a,5,6,6a,7-hexahydro-3a-hydroxy-6,9-dimethyl-3-methylenebenzo[*de*]chromen-4(2*H*,9a*H*)-one.

Stereumin E (**5**) was obtained as colorless powder. The HRESIMS data determined the molecular formula to be $C_{15}H_{18}O_4$ ($[M+H]^+$ at *m/z* 263.1285). The ^1H and ^{13}C NMR spectra (Table 3) indicated 15 C signals including one double bond (δ 124.4 and 151.2), one carboxyl group (δ 165.5) and one ketone group (δ 202.1). The data of δ_C 75.1 and δ_H 4.57 (*d*, 4.1) were indicative of ester groups, and further signals at δ_C 58.8, 60.2 and δ_H 3.24 (*d*, 4.9) indicated the presence of a three-membered ring which was composed of a CH–O–C unit. The total attribution for the observed signals was accomplished based on HMQC and HMBC correlations.

Additional HMBC correlations of 1-CH_3 (δ 1.97) with C-1, C-8a, C-2, H-3a with C-3b, C-4, 3b-CH_3 with C-3a, C-3b, H-4 with C-5, C-5a, H_β-5 with C-6, C-3b, C-5a, which together with the ^1H,^1H-COSY correlations between H-3a and H-8b afforded fragment **5a** (Fig. 2). Meanwhile, the correlations between H_α-7 and C-8, 6-CH_3 and C-8 (w), C-7, C-6 provided fragment **5b** (Fig. 2). Fragments

Table 3
^1H, ^{13}C and HMBC spectroscopic data for stereumin E (**5**)

Position	δ_H	δ_C	HMBC
1	\	124.4	\
2	\	165.5	\
3a	4.57 *d* (4.1)	75.1	3b, 4
3b	\	58.8	\
4	3.24 *d* (4.9)	60.2	5, 5a
5	2.36 *m*	29.2	6, 8b, 5a
	1.70 *m*		5a, 8b, 6, 3b
5a	2.38 *m*	40.3	6, 8b, 5a
6	1.64 *m*	38.1	8b
7	2.67 *dd* (4.8, 15.2)	52.4	5a, 8, 8a
	2.18 *dd* (12.0, 15.5)		8, 6-CH_3, 5a
8	\	202.1	\
8a	\	151.2	\
8b	2.42 *m*	40.0	\
1-CH_3	1.97 *s*	13.4	1, 8a, 2
3b-CH_3	1.54 *s*	19.7	3b, 3a
6-CH_3	1.04 *d* (6.5)	20.1	8(w), 7, 6, 5a

5a and **5b** were connected based on the correlations of H_β-7 with C-5a, and H-6 with C-8b. The relative configuration was deduced from ROESY experiments, in that H-4 was correlated with 6-CH_3, 6-CH_3 with H-5a, H-3a with H-8b, and 3b-CH_3 with H-6, H-8b (Fig. 2). The spectroscopic data established the structure of **5** to be (3a*R**,4*S**,5*S**,6*R**,8b*R**)-3a,3b,4a,5,5a,6,7,8b-octahydro-3,4-dioxa-1,3b,6-trimethyl-cyclopropa[*a*]phenalene-2,8-dione (Kawamura et al., 2000).

2.3. Nematicidal activities of compounds 1–5

The nematicidal activities of compounds **1**–**5** against *P. redivivus* were evaluated. Compounds **1**–**5** showed notable nematicidal activities at 400 mg l^{-1} (Table 4). Among the

Table 4
Effect of compounds **1–5** and avermectin on the mortality (%) of *P. redivivus*

Compounds	Concentrations (mg l^{-1})	*P. redivivus*	
		24 h	48 h
1	400	34.7	49.7
	200	31.6	35.1
2	400	55.1	79.5
	200	39.2	47.8
3	400	81.9	84.4
	200	33.8	47.2
4	400	89.3	94.9
	200	40.5	51.4
5	400	68.8	82.7
	200	27.4	35.5
Avermectin	400	30.7	67.2
	200	26.4	51.4
Control (5% acetone)		1.5	2.0

five compounds, compounds **3** and **4** exhibited potent activities comparable to the activity of the same amount of the standard nematocide avermectin, which killed 84.4% and 94.9% of *P. redivivus* at 400 mg l^{-1}, respectively, in 48 h. The control of 5% acetone just killed 1.5% and 2.0% the nematode at 24 h and 48 h, respectively. It is obvious that analogous compounds have similar activities: compounds **1** and **2** have similar activities while compounds **3** and **4** were similar. In spite of their overall structural similarity, the slight differences in functional groups contribute to the differences in nematicidal activities among the five compounds.

2.4. Conclusions

Secondary metabolites in fungi have much potential in their novel structures and nematicidal activities. Many nematicidal compounds have been found from fungi (Anke and Sterner, 1997). In our previous study, two new nematicidal aromatics were obtained from another strain of the genus *Stereum* (Li, 2006). The species in the *Stereum* are abundant, but only these compounds from the strain of *Stereum* were reported to possess nematicidal activities by far. It is suggested that more species and strains belonging to this genus should be screened to search for new sources of nematicidal substances.

3. Experimental

3.1. General

The NMR spectra were recorded on a Bruker DRX-500 spectrometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. HRESIMS and ESIMS data were obtained on VG Auto-Spec-3000 and Finnigan Trace DSQ mass spectrometer, respectively. The IR spectra were measured on a Paragon 1000 PC spectrometer. TLC was performed on plates precoated with Silica Gel G and Silica Gel CC used Silica Gel G 200–300 mesh (Qingdao Marine Chemical Inc., China). Sephadex LH-20 for CC was purchased from Amersham Biosciences.

3.2. Identification of fungal material and fermentations

The fungus used in this study was collected in Xishuangbanna, Yunnan Province, PR China. The mycelium of the basidiomycete was separated from its fruiting body and the voucher specimen was deposited in China Center for Type Culture Collection (the number of strain: CCTCC AF 207024).

DNA extraction, PCR and sequencing: The total DNA was isolated from fresh mycelium described by Turner et al. (1997). Primer pairs ITS4 and ITS5 (White et al., 1990), as well as LROR (Bunyard et al., 1994) and LR5 (Vilgalys and Hester, 1990) were used to amplify the complete ITS (including 5.8S) and partial large subunit (28S) sequence, respectively. The parameters for PCR amplifications were as follows: 3 min initial denaturation at 94 °C, followed by 30 cycles of 1 min denaturation at 94 °C, 1 min primer annealing at 50 °C, 90 s extension at 74 °C, and a final extension period of 7 min at 74 °C. The PCR products were purified with a commercial Kit (TaKaRa Biotechnology Co., Ltd.), and sequenced with aid of a LI-COR 4000L automatic sequencing system, using cycle sequencing with the ThermoSequenase-kit as described by Kindermann et al. (1998). BLAST searching was used to obtain similar sequences.

The fungus was grown in a shake culture (150 ml per 250 ml triangular flask) on a PDB medium consisting of glucose (20 g), potato (200 g, boiled and filtered) (per liter of water), and incubated for 8 days at 120 rpm under 25 °C. The culture was harvested for further study.

3.3. Extraction and isolation

The bioassay result showed that the $CHCl_3$ fraction was the active fraction with nematicidal activity. In further experiments, the bioassay guide was used in the isolation process for nematicidal compounds. The $CHCl_3$ extract (3 g) of the culture broth (15 l) was subjected to Silica Gel G CC (200–300 mesh; petroleum ether/acetone 20:1–2:1) to yield fractions A_1–A_8. Fraction A_2 was applied to a Silica Gel G CC (200–300 mesh; petroleum ether/EtOAc 20:1) and then purified by Sephadex LH-20 CC eluted with acetone to furnish compound **4** (8 mg). Fraction A_3 was purified further by Sephadex LH-20 CC eluted with acetone to give fraction $A_{3\text{-}2}$ and fraction $A_{3\text{-}2}$ was repeatedly subjected to Silica Gel G CC (200–300 mesh; petroleum ether/EtOAc 20:1) to obtain compound **3** (10 mg). Fraction A_4 was subjected to Silica Gel G CC (200–300 mesh; petroleum ether/acetone 20:1) to yield compound **5** (8 mg). Fraction A_7 was purified by Silica Gel G CC (200–300 mesh; petroleum ether/EtOAc 9:1–4:1) to yield fraction $A_{7\text{-}3}$ and further purification of fraction $A_{7\text{-}3}$ by Sephadex

LH-20 CC eluted with acetone to afford compound **2** (40 mg). Fraction A_8 was subjected to Silica Gel G column (200–300 mesh; petroleum ether/EtOAc 9:1–2:1) to give fraction $A_{8\text{-}5}$. Fraction $A_{8\text{-}5}$ was repeatedly purified by Silica Gel G CC (200–300 mesh; petroleum ether/acetone 10:1) to furnish compound **1** (15 mg).

3.4. Stereumin A (*1*)

Colorless needles; $[\alpha]_D^{22}$ + 142 (MeOH, *c* 0.60); IR (KBr) ν_{max} cm^{-1}: 3452, 2950, 1620, 1584, 1138, 1034; ESIMS: *m/z* 289 ($[M+Na]^+$); for NMR spectroscopic data, see Table 1.

3.5. Stereumin B (*2*)

Colorless cuboid crystals; $[\alpha]_D^{22}$ + 22 ($CHCl_3$, *c* 0.60); IR (KBr) ν_{max} cm^{-1}: 3400, 2936, 1728, 1676, 1582, 1034; ESIMS: *m/z* 265 ($[M-H]^-$); for NMR spectroscopic data, see Table 1.

3.6. Stereumin C (*3*)

Colorless powder; $[\alpha]_D^{22}$ + 120 ($CHCl_3$, *c* 0.60); IR (KBr) ν_{max} cm^{-1}: 3429, 2928, 1720(s), 1654, 1102, 1028; ESIMS: *m/z* 301 ($[M+Na]^+$); for NMR spectroscopic data, see Table 2.

3.7. Stereumin D (*4*)

Colorless powder; $[\alpha]_D^{22}$ + 131 ($CHCl_3$, *c* 1.60); IR (KBr) ν_{max} cm^{-1}: 3488, 2922, 1734, 1676, 1582, 1034; ESIMS: *m/z* 271 ($[M+Na]^+$), 519 ($[2M+Na]^+$); for NMR spectroscopic data, see Table 2.

3.8. Stereumin E (*5*)

Colorless powder; $[\alpha]_D^{22}$ + 176 ($CHCl_3$, *c* 0.60); IR (KBr) ν_{max} cm^{-1}: 3429, 2928, 1734(s), 1707(s), 1654, 1130; ESIMS: *m/z* 263 ($[M+H]^+$); for NMR spectroscopic data, see Table 3.

3.9. X-ray crystallographic data for *1*

$C_{15}H_{22}O_4$, $M_w = 266.33$, orthorhombic space group $P2_12_12_1$, $a = 6.7067(12)$ Å, $b = 14.330(3)$ Å, $c = 14.863(3)$ Å, $V = 1428.5(4)$ $Å^3$, $Z = 4$, $d = 1.238$ Mg/m^3. $F(000) = 576$, $\mu = 0.089$ mm^{-1}. A single crystal of dimensions $0.18 \times 0.12 \times 0.07$ mm was used for X-ray measurements. The intensity data of all unique reflections within the θ range 1.97–28.55° were collected at 298 K in a Bruker Apex II CCD Area Detector, using graphite monochromated Mo Kα ($\lambda = 0.71073$ Å) radiation. A total of 12,469 independent reflections were measured, and 3389 were considered to be observed ($|F|^2 \geq 2\sigma|F|^2$). All calculations were performed using the Crystal Structure crystallographic software package except for refinement, which was performed using SHELXL-97. CCDC 671092 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

3.10. X-ray crystallographic data for *2*

$C_{15}H_{22}O_4$, $M_w = 266.33$, orthorhombic space group $P2_12_12_1$, $a = 9.4412(10)$ Å, $b = 11.1335(12)$ Å, $c = 13.4732(14)$ Å, $V = 1416.2(3)$ $Å^3$, $Z = 4$, $d = 1.249$ mg/m^3. $F(000) = 576$, $\mu = 0.089$ mm^{-1}. A single crystal of dimensions $0.450 \times 0.392 \times 0.257$ mm was used for X-ray measurements. The intensity data of all unique reflections within the θ range 2.37–27.00° were collected at 293 K in a SMART APEX CCD diffractometer, using graphite monochromated Mo Kα ($k = 0.71073$ Å) radiation. A total of 8356 independent reflections were measured, and 1778 were considered to be observed ($|F|^2 \geqslant 2\sigma|F|^2$). Hydrogen atoms were fixed at calculated positions. The structure was solved by the direct method SHELX-97 and expanded using difference Fourier techniques, refined by the program and full-matrix least squares calculations. CCDC 671093 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

3.11. Nematicidal activity assay

The nematode *P. redivivus* was cultured on oatmeal medium (20 g of oatmeal in 80 ml of H_2O) at 25 °C for 7 days. The cultured nematodes were separated from the culture medium using the Baerman funnel technique (Gray, 1984). Compounds **1–5** were dissolved in acetone and then diluted to different concentrations (400 and 200 mg l^{-1}) with sterile H_2O. As a positive control, avermectin (Lynhi Fine Chemical Co. Ltd, Shijiazhuang, China) was used. At the same time, 5% acetone was used as negative control. Each treatment was replicated two times. The nematicidal activity against *P. redivivus* was assayed according to the method described in Li et al. (2005). The assay on nematicidal activity was repeated twice.

Acknowledgments

This work was partially supported by the National Basic Research Program of China (973 Program 2007CB411600), the Science and Technology Department of Yunnan Province (2005NG05 and 2005NG03), the Key Applied Foundation Program of Yunnan Province (1999C0001Z), the Natural Science Foundation of Yunnan Province (2004C0003Q). The authors thank School of Chemical Science and Technology, Yunnan University for the X-ray

analysis of compound **1** and the Analytical Department of Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences for the X-ray analysis of compound **2.**

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