JOURNAL OF NATURAL PRODUCTS

NOTE

pubs.acs.org/jnp

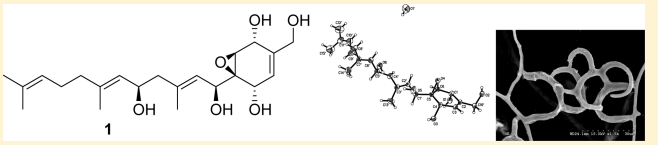
Arthrobotrisins A−C, Oligosporons from the Nematode-Trapping Fungus *Arthrobotrys oligospora*

Lu-Xia Wei, Hui-Xiang Zhang, Jian-Lin Tan, Yan-Sheng Chu, Nan Li, Hua-Xi Xue, Yan-Li Wang, Xue-Mei Niu,* Ying Zhang, and Ke-Qin Zhang*

Laboratory for Conservation and Utilization of Bio-Resources & Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan University, Kunming, 650091, People's Republic of China

Supporting Information

ABSTRACT: *Arthrobotrys oligospora* is a carnivorous fungus that can use mycelia trapping devices to capture their prey. Three novel oligosporons, named arthrobotrisins A−C (**1**−**3**), were isolated from *A. oligospora* and identified by spectroscopic analysis in combination with X-ray diffraction. This is the first time that the relative configuration of naturally occurring oligosporon metabolites has been fully determined. Compound **3** exhibited specific antibacterial activities.



Arthrobotrys oligospora , the first recognized nematode-trapping fungus, is the most commonly isolated and by far the most abundant nematode-trapping fungus in the environment.[1] Within a few hours of close contact with nematodes, the sparse mycelia of *A. oligospora* can spontaneously differentiate into three-dimensional network trapping devices, which then adhere to, penetrate, kill, and digest the nematodes.[2,3] Like carnivorous plants, *A. oligospora* exhibits a fascinating example of convergent evolution with the development of predatory organs and an increased capacity for predation under a low-nutrient environment. *A. oligospora* has been extensively studied as a potential biological control agent against nematode parasites of plants and animals.[4] Recently, the genome-sequencing of *A. oligospora* was accomplished.[5]

In 1993 and 1995, the research groups of Anke[6] and Rickards[7] reported unique antibiotics of the oligosporon group from strains of *A. oligospora* from The Netherlands and Australia, respectively. The skeletal features of these secondary metabolites are formed by alkylation of a polyketide-derived epoxy nucleus with a terpenoid-derived farnesyl unit. These compounds exhibited interesting biological activities, including nematicidal and antibacterial properties, and represented the most complex structural type of bioactive metabolites to be isolated and characterized from cultures of nematophagous fungi. However, all metabolites of this group were rather unstable, decomposing on storage even when refrigerated.[6,7] To date, the relative configuration of these metabolites has not been reported. Hybrid natural metabolites derived through mixed biosynthetic pathways have been evoking interest, as they embody diverse structural fragments.[8] The unique structures of these known oligosporons coupled with their interesting bioactivity profile caught our attention in view of our ongoing program on biologically active naturally occurring metabolites from *A. oligospora*.

A chemical investigation of *A. oligospora* fungal strain YMF 1.3170 led to the isolation of three novel metabolites of the oligosporon group, arthrobotrisins A−C (**1**−**3**). Their structures and relative configuration were elucidated on the basis of extensive NMR, MS, and X-ray crystallographic analysis. A chemotaxonomical relationship between this group of metabolites and the species is also revealed. The activity of the two major arthrobotrisins (**2**, **3**) against *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus* sp. 3.38, and *Bacillus* sp. 3.42 and the free living nematode *Panagrellus redivevus* is reported herein.

An EtOAc layer of the filtrate of the strain *A. oligospora* YMF 1.3170 was chromatographed repeatedly by column chromatography on macroporous resin, Sephadex LH-20 gel, silica gel, and RP-18, yielding three compounds.

Compounds **1**−**3**, obtained as colorless oils, all exhibited a quasi-molecular ion peak at m/z 429 $[M + Cl]^-$ in their negative ESI spectra and were assigned to a molecular formula of $C_{22}H_{34}O_6$, which was confirmed by HRESIMS and NMR spectrometric data (Table 1). The ^{1}H NMR, ^{13}C NMR, and DEPT spectra of **1**−**3** all displayed the presence of four tertiary methyl groups, three methylenes, one oxymethylene, five oxymethines, one oxygen-bearing quaternary carbon, four olefinic methines, and four olefinic quaternary carbons, which suggested that the basic skeleton of **1**−**3** was a farnesyl side chain connected to an epoxy-cyclohexenol moiety, with five hydroxyl groups as its substituents. Compounds **1**−**3** did not contain an acetyl group attached to CH_2OH at C-2, as is the case in all other known oligosporons. Their HSQC spectra enabled the assignment of all protons bonded with carbons. The coupling constants between H-3 and H-4 (ca. 5 Hz) in compounds **1**−**3** were the same as those for the known oligosporon group

Received: February 25, 2011
Published: May 13, 2011

Table 1. ^{1}H (400 MHz, acetone-d_6) and ^{13}C NMR (100 MHz) Data of Arthrobotrisins A (1), B (2), and C (3)

	arthrobotrisin A (1)		arthrobotrisin B (2)		arthrobotrisin C (3)	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	64.1, CH	4.23, brs	64.2, CH	4.22, d (8.4)	66.3	4.52, brd (6.7)
2	138.1, C		138.0, C		138.5	
3	123.2, CH	5.75, d (4.9)	122.5, CH	5.70, d (4.8)	122.4	5.66, dt (4.9, 1.4)
4	63. 5, CH	4.59, brt (4.8)	63.8, CH	4.57, brt (4.9)	64.3	4.58, t (4.8)
5	62.4, C		61.9, C		64.8	
6	58.5, CH	3.05 s	58.6, CH	3.06 s	60.1	3.21, d (2.5)
1′	67.4, CH	5.04, d (8.8)	68.0, CH	4.95, dd (8.5, 6.7)	68.3	4.86, dd (8.4, 6.5)
2′	126.5, CH	5.17, d (8.8)	125.1, CH	5.35, d (8.6)	124.8	5.42, d (8.7)
3′	139.3, C		141.7, C		142.0	
4′	49.3, CH_2	2.31, dd (12.7, 6.8)	77.5, CH	3.97, brt (6.9)	77.3	3.99, brt (6.6)
	2.16, dd[a]					
5′	67.4, CH	4.51, dd (15.2, 6.8)	35.0, CH_2	2.23, m	35.1	2.24, t (6.9)
6′	128.6, CH	5.14, t[a]	121.6, CH	5.14, brt (7.0)	121.8	5.18, t (6.9)
7′	137.6, C		137.1, C		137.0	
8′	40.5, CH_2	2.12, m[a]	40.4, CH_2	2.08, m[a]	40.5	2.07, m[a]
		2.02, m		2.00, m[a]		2.00, m[a]
9′	27.3, CH_2	2.15, m[a]	27.3, CH_2	2.09, m[a]	27.3	2.08, m[a]
		2.05, m		2.01, m[a]		2.01, m[a]
10′	125.2, CH	5.13, t[a]	125.2, CH_2	5.10, brt (6.8)	125.2	5.11, tt (6.8, 1.4)
11′	132.4, C		131.6, C		131.6	
12′	25.9, CH_3	1.67, s	25.8, CH_3	1.64, s	25.8	1.65, s
1-OH				4.31, d (8.5)		4.32, d (6.6)
2-CH_2OH	63.8, CH_2	4.10, ABd (13.8)	63.9, CH_2	4.13, ABd (13.8)	63.1	4.09, ABd (13.8)
		4.06, ABd (13.8)		4.06, ABd (13.8)		4.06, ABd (13.8)
4-OH				3.97, d[a]		3.97, d[a]
1′ -OH				4.27, d (7.3)		4.14, d (7.1)
3′ -CH_3	17.2, CH_3	1.71, s	12.1, CH_3	1.64, s	12.2	1.66, s
4′ -OH				3.82, d (3.6)		3.81, d (3.3)
7′ -CH_3	16.9, CH_3	1.70, s	16.5, CH_3	1.62, s	16.4	1.62, s
11′-CH_3	17.8, CH_3	1.61, s	17.8, CH_3	1.58, s	17.7	1.59, s

[a] Signal partially obscured.

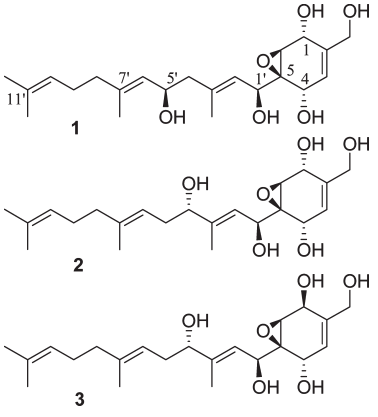


Figure 1. Arthrobotrisins A−C (1−3) from *A. oligospora* YMF 1.3170.

compounds, suggesting that the oxirane ring and the C-4 hydroxyl group in 1−3 are also oppositely oriented. Since the configuration of the oxirane ring in all the known oligosporon compounds isolated thus far from *A. oligospora* was exclusively β-oriented,[7] the oxirane ring and the hydroxyl group at C-4 in 1−3 were accordingly assumed to be of β- and α-configuration, respectively.

Comparison of the chemical shifts of 1 with the known compound oligosporol B[6,7] demonstrated that 1 was very similar to oligosporol B except that 1 had one more hydroxyl group and lacked the acetyl group. Detailed analysis of the $^{1}H-^{1}H$ coupling relationships and $^{1}H-^{13}C$ long-range correlations (Figure 1) in the 2D spectra of 1 led to the establishment of the extra hydroxyl group attached to C-5′ of the farnesyl chain.

The relative configuration of the farnesyl chain in 1 could not be determined on the basis of NMR data, although the obvious NOE correlations of H-5′ with two methyls at C-3′ and C-7′ in the NOESY spectrum indicated that H-5′ shared the same orientation as the two methyls at C-3′ and C-7′. Due to the instability of the compounds, attempts to prepare derivatives for further analysis of the stereochemistry of the remaining chiral centers with NMR spectrometry failed; acetylation of 1 with acetic anhydride in pyridine and synthesis of α-methoxy-α-trifluoromethylphenylacetate (MTPA) esters of 1 all resulted in extensive decomposition.

dx.doi.org/10.1021/np200187z |J. Nat. Prod. 2011, 74, 1526–1530

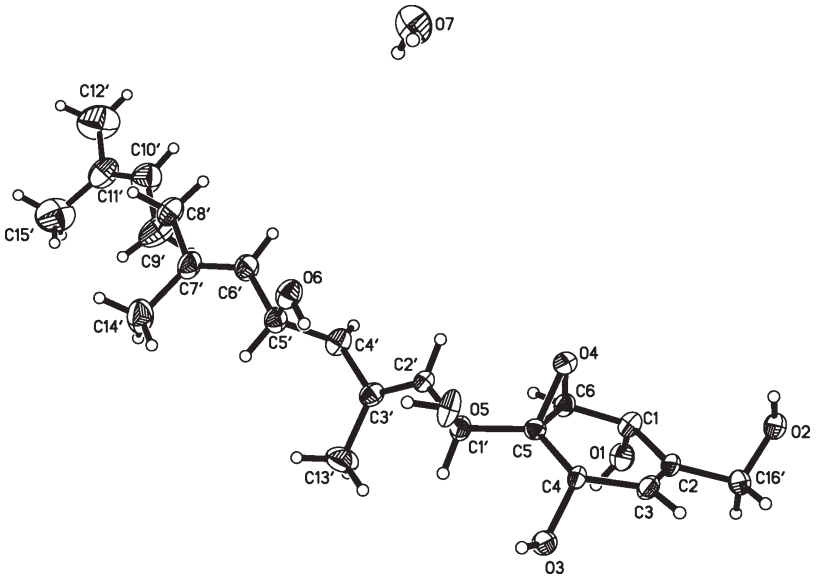


Figure 2. Single-crystal X-ray crystallographic structure of arthrobotrisins A (**1**).

A single crystal of **1** was obtained as a colorless crystal from a mixture of acetone and methanol (10:1) after several crystallization attempts. Its X-ray crystallographic analysis (Figure 2) was successfully carried out. The result unambiguously established not only the stereochemistry of C-1′ and C-5′ but also the complete structure and relative configuration of **1**. The farnesyl chain in the crystal structure of **1** shows a well-shaped *Z*-conformation between C-1′ and C-7′. Both hydroxyl groups at C-1′ and C-5′ were β-configured. Consequently, **1** was identified as (1*R*′,2*S*′,5*R*′,6*S*′)-1-((1*S*′,2*E*′,5*R*′,6*E*′)-1,5-dihydroxy-3,7,11-trimethyldodeca-2,6,10-trienyl)-4-(hydroxymethyl)-7-oxa-bicyclo[4.1.0]-hept-3-ene-2,5-diol and named arthrobotrisin A.

This is the first time that the relative configuration of a naturally occurring oligosporon metabolite has been fully determined, and it is also the first account of an X-ray structure analysis of the epoxy-cyclohexenol metabolites with a farnesyl side chain.

Comparison of the NMR data of **2** with those of **1** demonstrated a close resemblance, and the only difference was that **2** had one hydroxyl group at C-4′ instead of at C-5′ in **1**. This was further confirmed by a combination of $^{1}H-^{1}H$ coupling constants and long-range $^{1}H-^{13}C$ correlations in the 2D spectra of **2**. The stereochemistry of the C-4′ of the farnesyl chain in **2** was determined on the presumption that the farnesyl side chain in **2** preferred the similar well-shaped *Z*-conformations between C-1′ and C-7′ to those in **1**. The NOE correlations of H-4′ with H-2′ and H-6′ in the NOESY spectrum of **2**, together with the absence of the NOE correlation of H-4′ with 3′-CH_3, allowed us to establish the stereochemistry of H-4′ in **2** as the same β-configuration as those of H-2′ and H-6′ in **1**. Thus, the hydroxyl group at C-4′ in **2** was in the α-configuration. Compound **2** was established as (1*R*′,2*S*′,5*R*′,6*S*′)-1-((1*S*′,2*E*′,4*S*′,6*E*′)-1,4-dihydroxy-3,7,11-trimethyldodeca-2,6,10-trienyl)-4-(hydroxymethyl)-7-oxa-bicyclo-[4.1.0]hept-3-ene-2,5-diol and named arthrobotrisin B.

The NMR data of **3** showed similar features to those of **2** with slight differences in chemical shifts, indicating that they were one pair of stereoisomers. Detailed comparison of their NMR data revealed that the configuration of the hydroxyl group at C-1 in **3** and **2** was reversed. The stereochemistry of C-1 in **3** was elucidated as β-configuration, which was confirmed by comparison of the chemical shifts of H-1 and C-1 in **2** and **3** with those of two C-1 epimers, oligosporols B and A.[6,7] Thus, compound **3** was established as (1*R*′,2*S*′,5*S*′,6*S*′)-1-((1*S*′,2*E*′,4*S*′,6*E*′)-1,4-dihydroxy-3,7,11-trimethyldodeca-2,6,10-trienyl)-4-(hydroxymethyl)-7-oxa-bicyclo[4.1.0]hept-3-ene-2,5-diol and named arthrobotrisin C.

In contrast to the previously reported oligosporon group compounds, compounds **1**−**3** not only lacked the characteristic acetyl group at CH_2OH at the epoxy-cyclohexenol moiety but also contained an extra hydroxyl group in the farnesyl chain.

The metabolite profiles of five strains of the nematode-trapping fungus *A. oligospora* collected from different regions were determined. We were unable to obtain any oligosporon metabolites with an acetyl group on the cyclohexenol moiety as described in literature.[6,7] Most interestingly, the pair of C-1 epimers, compounds **2** and **3**, were obtained from all the *A. oligospora* strains tested. This result is similar to previous reports that another pair of C-1 epimers, oligosporols B and A, were isolated from strains of *A. oligospora* from The Netherlands and Australia. We have also cultured our five strains in different culture media and conditions that mimic their natural growth environments including low-nutrient conditions, low-nutrient conditions with nematodes, and adequate nutrient (Supporting Information). The occurrences of compounds **2** and **3** in the cultures were still not affected. In addition, the content of compound **2** increased with the addition of extra culture media (such as potato starch) in some cases.

The antimicrobial activities of **2** and **3** were determined by the agar diffusion assay method[9] against *Staphylococcus aureus* 3.17 and soil bacteria *Bacillus subtilis* 3.34, *Bacillus* sp. 3.38, and *Bacillus* sp. 3.42, using chloramphenicol as antibacterial reference. Compound **3** exhibited strong antibacterial activity against *B. subtilis* 3.34. Inhibition zones of 14.2 and 19.4 mm were produced by 25 μg and 50 μg of **3** on 6 mm filter disks, respectively. Under the same conditions, 10 μg of chloramphenicol produced an inhibition zone of 36.3 mm diameter. The minimal amount of **3** inhibiting the growth of *B. subtilis* 3.34 was determined to be 5 μg on 6 mm filter disks. Additionally, **3** exhibited moderate activity against *Bacillus* sp. 3.38 and *S. aureus* 3.17 with an inhibition zone diameter of 9.1 and 7.0 mm,

respectively, produced from 50 μg of **3**. Compound **2** was much less active than **3**. Inhibition zones of 4.7 and 8.0 mm against *Bacillus* sp. 3.38 by using 150 and 300 μg of **2** were observed.

Nematocidal activity was assessed in a larval development assay using the worms of the free living nematode *Panagrellus redivevus*.[10] Neither compound **2** or **3** was active at concentrations up to 200 μg/mL in the nematicidal assay, presumably due to the absence of the keto group at C-1.[7]

EXPERIMENTAL SECTION

General Experimental Procedures. Silica gel 60 (Merck, 230−400 mesh) was used for column chromatography. Column chromatography was performed on 200−300 mesh silica gel (Qingdao Marine Chemical Factory, P. R. China). Optical rotations were measured on a Horiba-SEAP-300 spectropolarimeter. UV spectral data were obtained on a Shimadzu-210A double-beam spectrophotometer. IR spectra were recorded on a Bruker-Tensor-27 spectrometer with KBr pellets. NMR experiments were carried out on either a Bruker AV-400 or a DRX-500 spectrometer with TMS as internal standard. MS were recorded on a VG-Auto-Spec-3000 spectrometer. High-resolution ESIMS data were measured on a Bruker Bio-TOF III electrospray ionization mass spectrometer. The TLC spots were detected by spraying the TLC plates with 20% (w/v) H_2SO_4 and then heating them on a hot plate.

Fungal Material. The strain *A. oligospora* YMF 1.03170 was isolated from a grapery in Jiuquan, Gansu, People's Republic of China, in 2003, and identified as *A. oligospora* by the morphological features of the conidiophores and the submerged hyphae and rates of growth.[1−3] The isolate was deposited in the strain collection of Laboratory for Conservation and Utilization of Bio-Resources & Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan University. After the conidia had developed on PDA slants in test tubes at 25 °C, the strain was kept at −30 °C as stock cultures.

Extraction and Isolation. A 42 L amount of culture broth of the strain *A. oligospora* YMF 1.3170 was separated by filtration into the mycelia and filtrate. The filtrate was concentrated to 2.5 L and extracted five times with equal volumes of ethyl acetate in 1:1 ratio. The EtOAc layer was combined and evaporated under reduced pressure to give a brown gum (25.5 g). This gum was loaded onto a macroporous resin column and eluted with H_2O/MeOH with decreasing polarity (10%−40%−70%−100% MeOH) to yield four fractions (A−D) based on TLC behavior. Fraction C (10.5 g), obtained on elution with 50−90% MeOH/H_2O, was further subjected to a Sephadex LH-20 gel column eluting with MeOH to yield six subfractions. Subfraction 4 (1.15 g) was further subjected to a silica gel column eluting with $CHCl_3$/MeOH (20:1) to yield **1** (17 mg). The rest of subfraction 4 was further separated on a Sephadex LH-20 gel column eluting with acetone to yield five fragments. Fragment 4 (0.56 g) was loaded onto a RP_{18} column, eluting with H_2O/MeOH (30−50%) with decreasing polarity to give **2** (200 mg). Fragment 3 (0.46 g) was repeatedly subjected to a Sephadex LH-20 gel column eluting with acetone, a RP_{18} column eluting with H_2O/MeOH (35−45%) with decreasing polarity, and a silica gel column eluting with $CHCl_3$/MeOH (35:1) with increasing polarity to obtain **3** (2 mg).

Arthrobotrisin A (***1***): colorless crystal; $[\alpha]_D^{23.5}$ −3.56 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 215.4 (3.84) nm; IR (KBr) ν_{max} 3414, 3268, 3000, 2968, 2916, 2856, 1727, 1662, 1639, 1629, 1452, 1385, 1287, 1204, 1180, 1166, 1121, 1084, 1051, 1033, 1003, 990, 950, 893, 870, 851, 830, 807, 747, 613, 586, 546, 531 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) see Table 1; ^{13}C NMR (acetone-d_6, 100 MHz) see Table 1; negative ESIMS *m/z* 429 $[M + Cl]^-$; negative HRESIMS *m/z* 429.2046 $[M + Cl]^-$ (calcd for $C_{22}H_{34}O_6Cl$, 429.2043).

Arthrobotrisin B (***2***): colorless oil; $[\alpha]_D^{23.5}$ −12.29 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.14); IR (KBr) ν_{max} 3424, 2966, 2922, 2856, 1657, 1638, 1631, 1460, 1451, 1442, 1433, 1383, 1271, 1172, 1109, 1087, 1019, 948, 740, 670, 580, 552 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) see Table 1; ^{13}C NMR (acetone-d_6, 100 MHz) see Table 1; negative ESIMS *m/z* 429 $[M + Cl]^-$; negative HRESIMS *m/z* 429.2044 $[M + Cl]^-$ (calcd for $C_{22}H_{34}O_6Cl$, 429.2043).

Arthrobotrisin C (***3***): colorless oil; $[\alpha]_D^{23.5}$ +19.36 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 205.2 (3.90); IR (KBr) ν_{max} 3423, 2967, 2924, 2856, 1657, 1639, 1630, 1441, 1384, 1261, 1167, 1104, 1076, 1021, 950, 896 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) see Table 1; ^{13}C NMR (acetone-d_6, 100 MHz) see Table 1; negative ESIMS *m/z* 429 $[M + Cl]^-$; negative HRESIMS *m/z* 429.2041 $[M + Cl]^-$, calcd 429.2047 for $C_{22}H_{34}O_6Cl$.

Antibacterial Assay. The target strains used for testing antibacterial activity were Gram-positive bacteria *Staphylococcus aureus* 3.17 and soil bacteria *Bacillus subtilis* 3.34, *Bacillus* sp. 3.38, and *Bacillus* sp. 3.42 using chloramphenicol as reference. The strains were preserved in 10% glycerol at −140 °C in the Culture Collection of the Laboratory for Conservation and Utilization of Bioresources, Yunnan University, Yunnan Province, P. R. China. The in vitro antibacterial activity was tested according to the disk dilution method reported in the literature.[9]

Nematode Worm Bioassay. The free-living nematode *P. redivevus* was relatively easy to cultivate and was chosen as the test nematode in our study. *P. redivevus* was cultured on oatmeal medium (oatmeal: 20 g, water: 80 mL) at 28 °C for 7 days. The cultured nematodes (mixed stages) were obtained from culturing medium using the Baermann funnel technique. The nematicidal activity was tested according to the previous report.[10]

ASSOCIATED CONTENT

Supporting Information. Spectroscopic data (1D and 2D NMR spectra) of compounds **1**−**3** and CIF file of X-ray crystallographic data of compound **1** are available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: 86-871-503-4878. Fax: 86-871-503-4838. E-mail: niuxm@yahoo.com; kqzhang111@yahoo.com.cn.

ACKNOWLEDGMENT

This work was sponsored by projects from Major State Basic Research Development Program (2009CB125905), National Natural Science Foundation of China (30870083 and 31070051), the Department of Science and Technology of Yunnan Province (2008CD068 and 2010GA011), the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry ([2009]1341), and the Young Academic and Technical Leader Raising Foundation of Yunnan Province (2009CI051).

REFERENCES

(1) Duddington, C. L. *Nature* **1954**, *173*, 500–501.
(2) Cooke, R. C. *Nature* **1963**, *197*, 205.
(3) Nordbring-Hertz, B. *Mycologist* **2004**, *18*, 125–133.
(4) Niu, X. M.; Zhang, K. Q. *Mycology* **2011**in press.
(5) Yang, J. K.; Wang, L.; Ji, X. L.; Feng, Y.; Li, X. M.; Zou, C. G.; Xu, J. P.; Ren, Y.; Mi, Q. L.; Wu, J. L.; Liu, S. Q.; Liu, Y.; Huang, X. W.; Wang, H. Y.; Niu, X. M.; Li, J.; Liang, L. M.; Ji, K. F.; Zhou, W.; Yu, Z. F.; Li, G. H.; Liu, Y. J.; Li, L.; Qiao, M.; Feng, L.; Zhang, K. Q. *PLoS Pathog.*, in revision.
(6) Stadler, M.; Sterner, O.; Anke, H. *Z. Naturforsch.* **1993**, *48c*, 843–850.

(7) Anderson, M. G.; Jarman, T. B.; Rickards, R. W. *J. Antibiot.* **1995**, *48*, 391–398.
(8) Mehta, G.; Singh, V. *Chem. Soc. Rev.* **2002**, *31*, 324–334.
(9) Chu, Y. S.; Niu, X. M.; Wang, Y. L.; Guo, J. P.; Pan, W. Z.; Huang, X. W.; Zhang, K. Q. *Org. Lett.* **2010**, *12*, 4356–4359.
(10) Niu, X. M.; Wang, Y. L.; Xue, H. X.; Li, N.; Wei, L. X.; Mo, M. H.; Zhang, K. Q. *J. Agric. Food Chem.* **2010**, *58*, 828–834.