Pestalpolyols A–D, Cytotoxic Polyketides from *Pestalotiopsis* sp. cr013

Authors Jing Li[1,2,3], Jin Xie[1,2], Yin-He Yang[1], Xiao-Lian Li[1], Ying Zeng[1], Pei-Ji Zhao[1]

Affiliations
[1] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China
[2] Life Science College, Southwest Forestry University, Kunming, China
[3] University of Chinese Academy of Sciences, Beijing, China

Key words
- *Pestalotiopsis* sp. cr013
- Amphisphaeriaceae
- pestalpolyol
- X-ray crystallography
- polyketide
- cytotoxic activity

Abstract

Four novel polyketides, named pestalpolyols A (**1**), B (**2**), C (**3**), and D (**4**), were isolated from solid fermentation products of *Pestalotiopsis* sp. cr013. Their structures were elucidated by extensive spectroscopic methods, including 1D and 2D nuclear magnetic resonance and high-resolution electrospray ionization mass spectrometry experiments, and the absolute configuration was confirmed by single-crystal X-ray diffraction analysis using the anomalous scattering of Cu Kα radiation. The inhibitory activities of compounds **1, 2**, and **4** against five human tumor lines were tested *in vitro*, and showed IC_{50} values 2.3–31.2 μM.

Supporting information available online at http://www.thieme-connect.de/products

received January 4, 2015
revised April 17, 2015
accepted June 7, 2015

Bibliography
DOI http://dx.doi.org/10.1055/s-0035-1546257
Published online July 30, 2015
Planta Med 2015; 81: 1285–1289 © Georg Thieme Verlag KG Stuttgart · New York · ISSN 0032-0943

Correspondence
Prof. Dr. Pei-Ji Zhao
State Key Laboratory of Phytochemistry and Plant Resources in West China
Kunming Institute of Botany
Chinese Academy of Sciences
132# Lanhei Road
Kunming 650201
China
Phone: + 86 8 71 65 22 31 11
Fax: + 86 87 15 15 02 27
zhaopeiji@mail.kib.ac.cn

Introduction

Nature produces a variety of different species, prokaryotes, and eukaryotes. Among them, fungi play a very important but yet mostly unexplored role [1]. *Pestalotiopsis* species (Amphisphaeriaceae) are widely distributed all over the world and some species of this genus may cause diseases on a variety of plants [2–5]. In recent years, *Pestalotiopsis*, as a highly creative genus of varied and abundant secondary metabolites, has gained considerable attention by chemists [6]. Some products from the genus might be important as drug lead compounds for the treatment of human diseases and control of plant diseases e.g. cytotoxic torreyanic acid [7], cytotoxic chloropestolide A [8], and antifungal isopestacin [9]. In order to search for new structural and active compounds, a strain, *Pestalotiopsis* sp. cr013, which was isolated from the aeciospores (spores produced in an aecium of the rust fungi, which spread to and infect the primary host [10]) of *Cronartium ribicola*, was investigated and several new compounds were obtained, of which two ambuic acid analogs were published recently [11]. This report describes four novel pestalpolyol structures (**Fig. 1**) and their activities.

Results and Discussion

The nucleotide sequences for the ITS1–5.8 S rDNA-ITS4 region of strain cr013 was registered in the GenBank with the accession number KM652628, and the strain was determined to be *Pestalotiopsis* sp. according to the ITS analysis.

Compound **1** was obtained as colorless crystals, and its molecular formula, $C_{28}H_{50}O_4$, was deduced by HR-ESI-MS as well as ^{13}C NMR coupled with distortionless enhancement by polarization transfer (DEPT) spectra. Based on the 2D NMR data, including the heteronuclear single quantum correlation (HSQC), heteronuclear multiple-bond correlation (HMBC), and correlation spectroscopy (COSY) spectra (**Table 1**), the gross structure of **1** was determined to be a straight chain polyketide containing poly-double bond, poly-hydroxy, and poly-methyl substituents [12, 13]. The ^1H NMR, COSY, and HMBC spectra (**Fig. 2**) showed structure **1**. The rotating-frame NOE spectroscopy (ROESY) experiment showed nuclear Overhauser effect (NOE) interactions between H-22/H-7 and between H-24/H-11. X-ray crystallography, with the final refinement on the Cu KR data resulting in a Flack parameter of − 0.1 (2), allowed an unambiguous assignment of the complete absolute configuration of **1** with 4*E*, 6*S*, 7*S*, 8*E*, 10*S*, 11*S*, 12*E*, 14*S*, 15*R*, 16*S*, and 18*S*

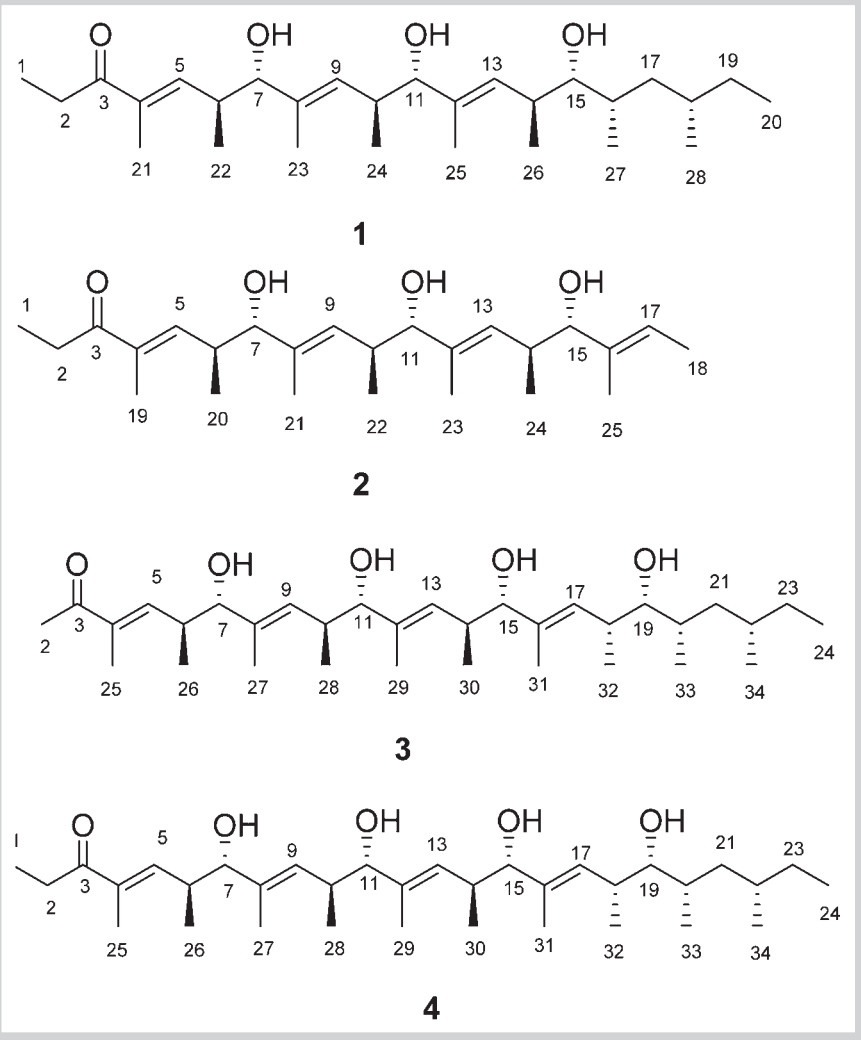

Fig. 1 Structures of compounds **1–4** from *Pestalotiopsis* sp. cr013.

configurations (**Fig. 3**). Compound **1** was named pestalpolyol A. Compound **2** was isolated as a colorless amorphous solid. The molecular formula was $C_{25}H_{42}O_4$ on the basis of HR-ESI-MS as well as ^{13}C-NMR coupled with DEPT spectra. According to the HMBC and COSY spectra (**Table 1**), the skeleton was almost the same as compound **1** expect for three carbons (**Fig. 1**). The 2D-NMR data (**Table 1**) led to the structure of **2**. A ROESY experiment showed correlations between H-20/H-7, H-7/H-9, H-9/H-11, H-22/H-11, H-11/H-13, H-13/H-15, and H-15/H-24. Based on similar NMR and specific rotation data of compounds **1** and **2**, and from a biogenetic perspective, the absolute configuration of **2** is proposed as shown in **Fig. 1** with 4*E*, 6*S*, 7*S*, 8*E*, 10*S*, 11*S*, 12*E*, 14*S*, 15*S*, and 16*E* configurations, and was named pestalpolyol B.

Compound **3** was isolated as a colorless amorphous solid. The molecular formula was assigned as $C_{33}H_{58}O_5$ on the basis of HR-ESI-MS as well as ^{13}C-NMR coupled with DEPT spectra. According to the HMBC and COSY spectra (**Table 2**), the skeleton was similar to compound **1** except for five carbons and a terminal $CH_3C{=}O$ (**Fig. 1**). A ROESY experiment showed correlations between H-26/H-7, H-7/H-9, H-9/H-11, H-11/H-28, H-11/H-13, H-13/H-15, H-15/H-30, H-15/H-17, H-17/H-19, H-19/H-18, H-19/H-20, and H-20/H-22. Comparison of NMR and specific rotation data of compounds **1** and **3**, and a biogenetic perspective, led to the absolute configuration of **3** proposed as shown in **Fig. 1** with 4*E*, 6*S*, 7*S*, 8*E*, 10*S*, 11*S*, 12*E*, 14*S*, 15*S*, 16*E*, 18*R*, 19*R*, 20*S*, and 22*S* configurations, named as pestalpolyol C.

Compound **4** was isolated as a colorless amorphous solid. The molecular formula was $C_{34}H_{60}O_5$ on the basis of HR-ESI-MS as well as ^{13}C-NMR and DEPT spectra. According to the HMBC and COSY spectra (**Table 2**), the skeleton of **4** was almost the same as compound **3**, except for one more carbon in a $CH_3CH_2C{=}O$ group (**Fig. 1**). The 2D-NMR data (**Table 2**) led to the structure of **4** proposed in **Fig. 1**. Comparison of NMR and specific rotation data of compounds **1** and **4**, and a biogenetic perspective, suggested the absolute configuration of **4** in **Fig. 1**, named as pestalpolyol D.

Compounds **1–4** were assayed for antifungal activity (*Gaeumannomyces graminis, Fusarinum moniliforme, Verticillium cinnabarium*, and *Phyricularia oryzae*) and antibacterial activity (*Pseudomonas solanacearum, Staphylococcus aureus*, and *Salmonella typhimurium* ATCC 6539), but did not show any inhibition activity at 100 µg/disk, nor any nematicidal activity. Compounds **1, 2**, and **4** were assayed for cytotoxicity. Compound **1** showed IC_{50} values of 10.4 µM (HL-60), 11.3 µM (SMMC-7721), 2.3 (A-549), 13.7 µM (MCF-7), and 12.4 µM (SW480). Compound **2** showed an IC_{50} value of 10.6 µM (A-549). Compound **4** showed IC_{50} values of 15.7 µM (HL-60), 31.2 µM (SMMC-7721), 10.7 µM (A-549), 23.7 µM (MCF-7), and 21.4 µM (SW480). The IC_{50} values of compounds **1** and **4** were similar to those of related compounds [13].

Table 1 [1] H-NMR (600 MHz, δ in ppm, mult. J in Hz), [13] C-NMR (150 MHz, δ in ppm), and HBMC data of **1** and **2**.

Pos.	1*			2**		
	δ_H	δ_C	HMBC (H→C)	δ_H	δ_C	HMBC (H→C)
1	1.08, t, (7.3)	9.4, q	C-2, C-3	1.09, t, (6.0)	9.5, q	C-2, C-3
2	2.79, m	31.3, t	C-1, C-3	2.73, q, (7.2)	30.9, t	C-1, C-3
3	–	205.1, s	–	–	202.5, s	–
4	–	137.7, s	–	–	136.9, s	–
5	6.72, dd, (1.1, 9.4)	148.2, d	C-3, C-4, C-6, C-7, C-21, C-22	6.98, d, (9.4)	147.4, d	C-3, C-6, C-7, C-19, C-20
6	2.82, m	38.4, d	C-4, C-5, C-7, C-22	3.07, m	38.7, d	C-4, C-5, C-7, C-20
7	3.86, d, 8.6	83.8, d	C-5, C-6, C-8, C-9, C-22, C-23	4.25, d, (7.7)	82.5, d	C-5, C-6, C-8, C-9, C-19, C-20
8	–	137.6, s	–	–	137.5, s	–
9	5.33, d, (1.4)	133.9, d	C-10, C-11, C-23, C-24	5.88, d, (9.3)	132.7, d	C-7, C-10, C-21, C-22
10	2.68, m	37.1, d	–	2.99, m	37.13, d	C-8, C-9, C-11, C-22
11	3.72, d, (8.6)	84.1, d	C-10, C-12, C-13, C-24, C-25	4.22, d, (7.3)	82.4, d	C-10, C-12, C-13, C-22, C-23
12	–	137.4, s	–	–	138.4, s	–
13	5.34, d, (1.5)	133.5, d	C-14, C-16, C-25	5.82, d, (9.1)	131.7, d	C-11, C-14, C-23, C-24
14	2.68, m	37.0, d	–	2.92, m	37.06, d	C-12, C-13, C-15, C-24
15	3.21, dd, (3.4, 7.9)	79.0, d	C-13, C-14, C-16, C-17, C-26, C-27	4.02, d, (8.4)	83.1, d	C-13, C-14, C-16, C-17, C-24, C-25
16	1.47, m, overlap	32.8, d	C-15, C-17, C-18	–	138.9, s	–
17	1.47, m, overlap	42.7, t	overlap	5.58, q, (6.6)	121.8, d	C-15, C-18, C-25
	1.04, m		C-15, C-16, C-19, C-27, C-28			
18	1.79, m	33.2, d	C-17	1.60, d, (6.6)	13.6, q	C-16, C-17
19	1.43, m	30.6, t	C-17, C-18, C-20, C-28	2.04, s	12.4, q	C-3, C-4, C-5
	1.14, m		C-17, C-18, C-20, C-28			
20	0.94, m, overlap	11.5, q	overlap	1.09, t, (6.0)	17.6, q	C-5, C-6, C-7
21	1.84, d, (1.3)	12.0, q	C-3, C-4, C-5	1.94, s	13.0, q	C-7, C-8, C-9
22	0.92, m, overlap	17.97, q	overlap	1.14, d, (6.7)	18.9, q	C-9, C-10, C-11
23	1.712, d, (1.2)	11.9, q	C-7, C-8, C-9	1.93, s	12.5, q	C-11, C-12, C-13
24	0.85, d, (6.8)	18.01, q	C-9, C-10, C-11	1.00, d, (6.7)	18.6, q	C-13, C-14, C-15
25	1.710, d, (1.2)	11.9, q	C-11, C-12, C-13	1.78, s	11.7, q	C-15, C-16, C-17
26	0.91, m, overlap	17.0, q	overlap	–	–	–
27	0.92, m, overlap	14.1, q	overlap	–	–	–
28	0.88, m, overlap	20.2, q	–	–	–	–

* The NMR data obtained in CD_3OD; ** the NMR data obtained in C_5D_5N

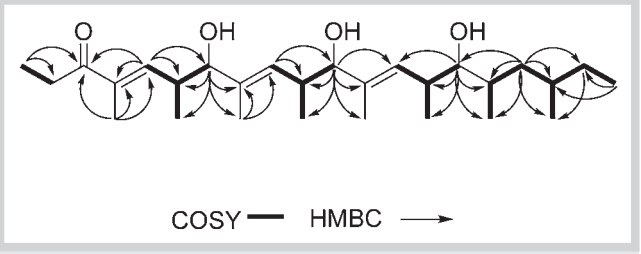

Fig. 2 Observed COSY and key HMBC correlations for compound **1**.

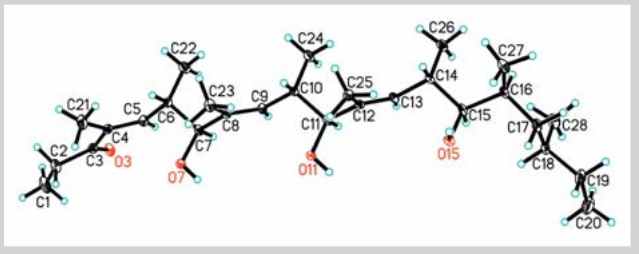

Fig. 3 Crystal X-ray structure of **1**. (Color figure available online only.)

Materials and Methods

General experimental procedures

The optical rotations were measured with a Jasco DIP-370 digital polarimeter. UV spectra were recorded on a Shimadzu UV-2401PC spectrophotometer. The NMR spectra were obtained with an Avance III 600 spectrometer. The ESI and HR-ESI-MS were recorded on Finnigan LCQ-Advantage and VG Auto-Spec-3000 mass spectrometers, respectively. Column chromatography was performed on silica gel G, silica gel GF254, silica gel 200–300 mesh (Qingdao Marine Chemical Factory), silica gel H (Merck), and Sephadex LH-20 (Amersham Pharmacia). Cisplatin (99% purity) was purchased from Sigma-Aldrich.

Microbial material

The aeciospores of *C. ribicola* were collected from *Pinus armandii* Franch. (Pinaceae) in Kunming, Yunnan Province, People's Republic of China, in March 2013, and identified by Dr. En-De Liu of Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. KUN1253509) was deposited at the Herbarium of Kunming Institute of Botany (KUN), Chinese Academy of Sciences. The aeciospores were incubated on distilled filter paper at 25 °C and cultured until a colony or mycelium appeared. After being cultured for about one week, a strain was isolated from the aeciospores, identified as *Pestalotiopsis* sp. (strain number cr013), and is preserved in the Kunming Institute of Botany, Chinese Academy of Science, Kunming, China. *Pestalotiopsis* sp. cr013 (30 L) was cultured on improved Fries culture agar (KH_2PO_4

Table 2 [1] H-NMR (600 MHz, δ in ppm, mult. J in Hz), [13] C-NMR (150 MHz, δ in ppm), and HBMC data of **3** and **4**.

Pos.	3*			4*		
	δ_H	δ_C	HMBC (H→C)	δ_H	δ_C	HMBC (H→C)
1	–	–	–	1.09, m	9.5, q	C-2, C-3
2	2.35, s	26.0, q	C-3, C-4, C-5	2.73, m	30.9, t	C-1, C-3
3	–	200.0, s	–	–	202.5, s	–
4	–	137.4, s	–	–	136.9, s	–
5	6.98, d, (9.4)	148.9, d	C-3, C-6, C-7, C-25, C-26	6.97, d, (9.4)	147.4, d	C-3, C-7, C-25, C-26
6	3.06, m	38.8, d	C-4, C-5, C-7, C-26	3.07, m	38.7, d	C-4, C-5, C-7, C-26
7	4.22, d, (7.8)	82.4, d	C-5, C-6, C-8, C-9, C-27, C-28	4.25, d, (7.8)	82.5, d	C-5, C-6, C-8, C-9, C-27, C-28
8	–	138.3, s	–	–	137.5, s	–
9	5.83, m	132.8, d	C-10, C-11, C-27, C-28	5.83, m	132.7, d	C-10, C-11, C-27, C-28
10	2.97, m	37.1, d	C-9, C-11, C-12, C-28	2.99, m	37.13, d	C-9, C-12, C-28
11	4.15, d, (7.7)	82.9, d	overlap	4.16, d, (7.6)	82.4, d	overlap
12	–	137.9, s	–	–	138.4, s	–
13	5.80, m	131.9, d	C-14, C-29, C-30	5.87, m	131.7, d	C-14, C-29, C-30
14	2.97, m	36.7, d	C-12, C-13, C-15, C-30	2.92, m	37.06, d	C-12, C-16, C-30
15	4.15, d, (7.7)	82.6, d	overlap	4.16, d, (7.6)	82.8, d	overlap
16	–	137.6, s	–	–	138.9, s	–
17	5.85, m	131.8, d	C-18, C-19, C-31, C-32	5.87, m	131.8, d	C-18, C-31, C-32
18	2.88, m	36.7, d	C-16, C-17, C-19, C-32	2.86, m	36.86, d	C-16, C-17, C-19, C-32
19	3.49, dd, (7.4, 3.4)	77.9, d	C-17, C-21, C-32, C-33	3.49, dd, (7.1, 3.4)	78.0, d	C-17, C-32, C-33
20	1.91, m	33.1, d	–	1.90, m	33.2, d	overlap
21	1.68, dt, (13.5, 6.9)	42.5, t	C-19, C-20, C-23, C-33, C-34	1.68, dt, (13.5, 6.9)	42.5, t	C-19, C-20, C-23, C-33, C-34
	1.17, dd, (13.5, 6.9)		C-19, C-20, C-23, C-33, C-34	1.16, m		C-23, C-33, C-34
22	1.55, td, (13.6, 6.8)	32.1, d	C-21, C-23, C-34, C-24	1.56, m	32.1, d	C-21, C-23, C-24, C-34
23	1.41, dq, (12.0, 7.2)	30.0, t	C-21, C-22, C-34, C-24	1.41, m	30.0, t	C-24, C-34
	1.07, m		–	1.09, m		overlap
24	0.85, t, (7.4)	12.3, q	C-22, C-23	0.87, t, (7.4)	12.0, q	C-22, C-23
25	2.03, s	12.2, q	C-3, C-4, C-5	2.04, s	12.5, q	C-3, C-4, C-5
26	1.06, d, (7.1)	17.5, q	148.9, 82.4, 38.8	1.07, m	17.6, q	overlap
27	1.93, s	12.9, q	overlap	1.94, s	12.9, q	overlap
28	1.08, m	18.7, q	overlap	1.13, m	18.9, q	overlap
29	1.93, s	12.6, q	overlap	1.93, s	12.5, q	overlap
30	1.08, m	18.7, q	overlap	1.12, m	18.6, q	overlap
31	1.89, s	12.4, q	C-15, C-16, C-17	1.90, s	11.7, q	C-15, C-16, C-17
32	1.08, m	18.6, q	overlap	1.07, m	18.77, q	overlap
33	1.13, d, (6.7)	14.8, q	C-19, C-20, C-21	1.11, m	14.8, q	C-19, C-20, C-21
34	0.89, d, (6.4)	20.3, q	C-21, C-22, C-23	0.87, d, 6.4	20.3, q	C-21, C-22, C-23

* The NMR data obtained in C_5D_5N

1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, NaCl 0.1 g, $CaCl_2 \cdot 2H_2O$ 0.13 g, saccharose 20.0 g, ammonium tartrate 5.0 g, yeast extract 1.0 g, NH_4NO_3 1.0 g, distilled water 1 L, and agar 15.0 g; pH was natural) dish at a temperature of 25 °C for 21 days.

Extraction and isolation

The solid fermentation products were cut into small pieces and extracted exhaustively with 30.0 L of mixture solution (EtOAc/MeOH/HAc, 80 : 15 : 5, v/v/v) three times (3 × 30 L) to obtain the rude extract. The extract were dissolved in water (500 mL), and extracted with 1000 mL EtOAc five times. The dried ethyl acetate extract (30.1 g) was subjected to a column of silica gel G (200–300 mesh, 6.5 × 45 cm, 250 g) using a $CHCl_3$-MeOH (100 : 0, 100 : 10, 90 : 10, 80 : 20, 0 : 100, each 4 L, 25 mL/min) gradient solvent system to produce five fractions (Fr. 1–Fr. 5). Fr. 1 (9.223 g) was placed in a column of silica gel (200–300 mesh, 3.5 × 43 cm, 80 g) and eluted with petroleum ether-EtOAc (100 : 10, 80 : 20, 70 : 30, 60 : 40, each 3 L, 15 mL/min) and a $CHCl_3$-MeOH (200 : 10, 100 : 10, 80 : 20, 0 : 100, each 3 L, 15 mL/min) gradient solvent system to yield fractions of Fr. 1.1–Fr. 1.11. Fr. 1.7 (534.8 mg) was subjected to a column of silica gel (GF254, 2.5 × 43 cm, 50 g) using a petroleum ether-acetone (100 : 7, 100 : 10, 80 : 20) gradient solvent system to give fractions of Fr. 1.7.1–Fr. 1.7.6. The Fr. 1.7.1 (112.3 mg) was purified by Sephadex LH-20 (MeOH, 2.5 × 120 cm, 1 mL/min) to produce two fractions (Fr. 1.7.1.1 and Fr. 1.7.1.2). Fr. 1.7.1.1 (72.8 mg) was subjected to a column of silica gel (GF254, 2.5 × 43 cm, 20 g) using a $CHCl_3$-acetone (100 : 6, 100 : 10, 80 : 20) gradient solvent system to produce **1** (55.5 mg). Fr. 2 (3.233 g) was placed in a column of silica gel (200–300 mesh, 3.5 × 43 cm, 80 g) and eluted with a $CHCl_3$-MeOH (20 : 1, 10 : 1, 9 : 1, 8 : 2, 7 : 3, each 1.5 L, 15 mL/min) gradient solvent system to yield fractions of Fr. 2.1–Fr. 2.5. Fr. 2.1 (322.1 mg) was placed in a column of silica gel (GF254, 2.5 × 43 cm, 30 g) using a petroleum ether-acetone (9 : 1, 7 : 3) gradient solvent system to give fractions of Fr. 2.1.1–Fr. 2.1.2. Fr. 2.1.1 (162.4 mg) was isolated by Sephadex LH-20 (MeOH, 2.5 × 120 cm, 1 mL/min) and a column of silica gel (GF254, 2.5 × 43 cm, 20 g) using $CHCl_3$-acetone (80 : 20, 70 : 30, 0 : 100) with formic acid (0.3%) to give fractions of Fr. 2.1.1.1.1–Fr. 2.1.1.1.3. At last, Fr. 2.1.1.1.3 (22.1 mg) was purified by semipreparative HPLC (LC3000 Semipreparation Gradient HPLC System, China, C_{18}, 5 µm, 254 nm, MeOH-H_2O = 90 : 10, flow rate 3.0 mL/min) to yield **3** (2.5 mg) and **4** (4.5 mg). Fr. 2.4

(286.3 mg) was purified by Sephadex LH-20 eluting with $CHCl_3$-MeOH (1 : 1, 3 × 150 cm, 1 mL/min) to produce two fractions (Fr. 2.4.1 and Fr. 2.4.2). Fr. 2.4.1 (198.8 mg) was isolated by Sephadex LH-20 (MeOH, 2.5 × 120 cm, 1 mL/min) to produce two fractions (Fr. 2.4.1.1 and Fr. 2.4.1.2). Fr. 2.4.1.1 (150.0 mg) was first purified using semipreparative HPLC (LC3000 Semipreparation Gradient HPLC System, China, C_{18}, 5 µm, 254 nm, MeOH-H_2O = 90 : 10, flow rate 3.0 mL/min) and then was isolated by a silica gel (GF254, 2.5 × 43 cm, 20 g) using petroleum ether-acetone (8 : 2) to produce **2** (2.0 mg).

Pestalpolyol A (**1**): Colorless crystals; m. p. 117–118 °C; $[\alpha]_D^{26}$ = + 4.56 (*c* = 0.13, MeOH); UV (MeOH) λ_{max} (log ε): 233 (4.03); IR (KBr) ν_{max}: 3428, 2962, 1673, 1630, 1454, 1385, 1036, 1019 cm^{-1}; NMR data see **Table 1**; ESI-MS: 473 [M + Na]$^+$; HR-ESI-MS: 473.3607 ([M + Na]$^+$, calcd. 473.3607).

Crystal data: All single-crystal X-ray diffraction data were collected at 100 (2) K on a Bruker APEX DUO with Cu Kα radiation (λ = 1.54178 Å). The structures were solved by direct methods (SHELXS-97) and refined using full-matrix least-squares difference Fourier techniques. $C_{28}H_{50}O_4$, M_r = 450.68, orthorhombic, a = 11.0339(5) Å, b = 11.5162(6) Å, c = 44.838(2) Å, α = 90.00°, β = 90.00°, γ = 90.00°, *V* = 5697.6(5) Å^3, *T* = 100(2) K, space group *P*212121, *Z* = 8, µ(CuKα) = 0.529 mm^{-1} 27477 reflections measured, 9921 independent reflections (R_{int} = 0.0752). The final R_1 values were 0.0709 [I > 2σ(I)]. The final wR(F^2) values were 0.1848 [I > 2σ(I)]. The final R_1 values were 0.0932 (all data). The final wR(F^2) values were 0.1983 (all data). The goodness of fit on F^2 was 1.075. Flack parameter = − 0.1(2). The Hooft parameter was 0.05(11) for 4158 Bijvoet pairs. Crystallographic data (excluding structure factors) have been deposited at the Cambridge Crystallographic Data Centre under the reference number CCDC 1020104. Copies of the data can be obtained free of charge on application to the CCDC, 12 Union Road, Cambridge CB2 IEZ, UK. Fax: + 44-(0)1223–336033 or e-mail: deposit@ccdc.cam.ac.uk.

Pestalpolyol B (**2**): Colorless amorphous solid; m. p. 195–196 °C; $[\alpha]_D^{20}$ = + 2.81 (*c* = 0.07, MeOH); UV (MeOH) λ_{max} (log ε): 233 (4.08); IR (KBr) ν_{max}: 3408, 2968, 1675, 1642, 1453, 1373, 1020 cm^{-1}; NMR data see **Table 1**; ESI-MS: 429 [M + Na]$^+$; HR-ESI-MS: 429.2975 ([M + Na]$^+$, calcd. 429.2981).

Pestalpolyol C (**3**): Colorless amorphous solid; $[\alpha]_D^{26}$ = + 17.05 (*c* = 0.07, MeOH); UV (MeOH) λ_{max} (log ε): 233 (4.20); NMR data see **Table 2**; ESI-MS: 557 [M + Na]$^+$; HR-ESI-MS: 557.4189 ([M + Na]$^+$, calcd. 557.4182).

Pestalpolyol D (**4**): Colorless amorphous solid; $[\alpha]_D^{26}$ = + 19.70 (*c* = 0.22, MeOH); UV (MeOH) λ_{max} (log ε): 232 (4.19); NMR data see **Table 2**; ESI-MS: 571 [M + Na]$^+$; HR-ESI-MS: 571.4344 ([M + Na]$^+$, calcd. 571.4338).

Biological activities

The antifungal activities were assayed against phytopathogenic fungi (*G. graminis, F. moniliforme, V. cinnabarium*, and *P. oryzae*) and antibacterial activities were assayed against *P. solanacearum, S. aureus*, and *S. typhimurium* ATCC 6539 using a disk diffusion method [14]. The method of nematicidal activity against *Panagrellus redivivus* and *Caenorhabditis elegans* was based on the literature [15]. Antitumor activity was measured by the MTT method [16]. Five cell lines were selected for testing (leukemia cell line HL-60, hepatocarcinoma cell line SMMC-7721, lung adenocarcinoma cell line A-549, breast cancer cell line MCF-7, and colon cancer cell line SW480). Cisplatin was used as a positive control and displayed IC_{50} values of 1.3 µM (HL-60), 6.7 µM (SMMC-7721), 6.2 µM (A-549), 16.3 µM (MCF-7), and 12.9 µM (SW480).

Supporting information

Supplementary 1D and 2D NMR spectroscopic data of compounds **1**–**4** are available as Supporting Information.

Acknowledgements

This work was supported by the National Basic Research Program of China (973 program, 2013CB127505), the NSFC (31170061, 31260177), and the Applied Basic Research Foundation of Yunnan Province (2013FA018). We acknowledge the Department of Instrumental Analysis of Kunming Institute of Botany for measuring the optical rotations, UV, NMR, and mass spectra.

Conflict of Interest

There are no conflicts of interest of any authors with respect to this work.

References

1 *Cole RJ, Schweickert MA.* Handbook of secondary fungal metabolites, Volumes I–III. San Diego, USA: Academic Press; 2003
2 *Hopkins KE, McQuilken MP.* Characteristics of *Pestalotiopsis* associated with hardy ornamental plants in the UK. Eur J Plant Pathol 2000; 106: 77–85
3 *Taylor JE, Crous PW, Palm ME.* Foliar and stem fungal pathogens of Proteaceae in Hawaii. Mycotaxon 2001; 78: 449–490
4 *Keith LM, Velasquez ME, Zee FT.* Identification and characterization of *Pestalotiopsis* spp. causing scab disease of guava, *Psidium guajava*, in Hawaii. Plant Dis 2006; 90: 16–23
5 *Maharachchikumbura SS, Guo LD, Chukeatirote E, Bahkali AH, Hyde KD.* A multi-locus backbone tree for *Pestalotiopsis*, with a polyphasic characterization of 14 new species. Fungal Divers 2011; 50: 167–187
6 *Xu J, Ebada SS, Proksch P. Pestalotiopsis* a highly creative genus: chemistry and bioactivity of secondary metabolites. Fungal Divers 2010; 44: 15–31
7 *Lee JC, Strobel GA, Lobkovsky E, Clardy J.* Torreyanic acid: a selectively cytotoxic quinone dimer from the endophytic fungus *Pestalotiopsis microspora*. J Org Chem 1996; 61: 3232–3233
8 *Liu L, Li Y, Liu SC, Zheng ZH, Chen XL, Zhang H, Guo LD, Che YS.* Chloropestolide A, an antitumor metabolite with an unprecedented spiroketal skeleton from *Pestalotiopsis fici*. Org Lett 2009; 11: 2836–2839
9 *Strobel G, Ford E, Worapong J, Harper JK, Arif AM, Grant DM, Fung PC, Ming Wah Chau R.* Isopestacin, an isobenzofuranone from *Pestalotiopsis microspora*, possessing antifungal and antioxidant activities. Phytochemistry 2002; 60: 179–183
10 *Dalton DT, Postman JD, Hummer KE.* Comparative infectivity of *Cronartium ribicola* aeciospores and urediniospores in genotypes of *Ribes nigrum*. Plant Dis 2010; 94: 461–464
11 *Xie J, Li J, Yang YH, Chen YH, Zhao PJ.* Two new ambuic acid analogs from *Pestalotiopsis* sp. cr013. Phytochem Lett 2014; 10: 291–294
12 *Cimino G, Sodano G, Spinella A.* Aglajne-1, a polypropionate metabolite from the opisthobranch mollusk *Aglaja depicta*: Determination of carbon-carbon connectivity via long-range ^{1}H-^{13}C couplings. Tetrahedron Lett 1985; 26: 3389–3392
13 *Kasai Y, Komatsu K, Shigemori H, Tsuda M, Mikami Y, Kobayashi J.* Cladionol A, a polyketide glycoside from marine-derived fungus *Gliocladium* species. J Nat Prod 2005; 68: 777–779
14 *Espinel-Ingroff A, White T, Pfaller MA.* Antifungal agents and susceptibility tests. In: Murray PR, editor. Manual of clinical microbiology, 7th edition. Washington DC: American Society for Microbiology; 1999: 1640–1652
15 *Li GH, Duan M, Yu ZF, Li L, Dong JY, Wang XB, Guo JW, Huang R, Wang M, Zhang KQ.* Stereumin A–E, sesquiterpenoids from the fungus *Stereum* sp. CCTCC AF 207024. Phytochemistry 2008; 69: 1439–1445
16 *Su J, Zhao PJ, Kong LM, Li XY, Yan JM, Zeng Y, Li Y.* Trichothecin induces cell death in NF-κB constitutively activated human cancer cells via inhibition of IKKβ phosphorylation. PLoS One 2013; 8: e71333