Tetrahedron xxx (2014) 1–6

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

New polyesters from *Talaromyces flavus*

Jun-Wei He [a,†], Zhen-Qiang Mu [a,†], Hao Gao [a,*], Guo-Dong Chen [a], Qin Zhao [a], Dan Hu [a], Jing-Zu Sun [c], Xiao-Xia Li [a], Yan Li [b], Xing-Zhong Liu [c], Xin-Sheng Yao [a,*]

[a] Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, People's Republic of China
[b] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China
[c] State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100190, People's Republic of China

ARTICLE INFO

Article history:
Received 30 July 2013
Received in revised form 15 February 2014
Accepted 24 February 2014
Available online xxx

Keywords:
Talaromyces flavus
Talapolyesters A–F
15G256 Polyesters
Wetland soil-derived fungus
Cytotoxicity

ABSTRACT

Six new polyesters, talapolyesters A–F (**1**–**4**, **14**, and **16**), together with 11 known ones 15G256ν (**5**), 15G256ν-me (**6**), 15G256π (**7**), 15G256β-2 (**8**), 15G256α-2 (**9**), 15G256α-2-me (**10**), 15G256ι (**11**), 15G256β (**12**), 15G256α (**13**), 15G256α-1 (**15**), and 15G256ω (**17**), were isolated from the wetland soil-derived fungus *Talaromyces flavus* BYD07-13, and their structures were determined by NMR and MS spectroscopic data. Among them, **1**–**4** and **16** were assembled in a different manner from that of the known 256 polyesters. All the polyesters are composed of (*R*)-2,4-dihydroxy-6-(2-hydroxypropyl)benzoic acid and (*R*)-3-hydroxybutyric acid/(*S*)-3,4-dihydroxybutyric acid residues. The absolute configurations of the residues were determined by alkaline hydrolysis. The cytotoxicity against five tumor cell lines of these compounds was examined, and a tight structure–activity relationship is proposed.

1. Introduction

15G256 Polyesters were originally isolated from the fermentation extract of the fungus *Hypoxylon oceanicum*,[1,2] which were also found from other genus fungi, such as *Penicillium*,[3,4] *Acremonium*,[5] *Albatrellus*,[6] *Talaromyces*,[7,8] *Scedosporium*,[9] *Calcarisporium*,[10] and *Callophyllum*[11] species. As proposed by Schlingmann,[1] 15G256 polyesters are biosynthetically assembled by alternately linking 2,4-dihydroxy-6-(2-hydroxypropyl)benzoic acid and 3-hydroxybutyric acid moieties. The compounds are members of a group of cyclic or linear polyesters containing both 2,4-dihydroxy-6-(2-hydroxypropyl)benzoic acid or its derivatives (B-unit) and 3-hydroxybutyric acid or its derivatives (A-unit). So far, only eight cyclic and eight linear ones have been identified from nature.[1–11] It is worth mentioning that 15G256ι and 15G256β were synthesized by Barrett and co-workers in 2008.[12] In our ongoing study of bioactive secondary metabolites from fungi,[13–18] a chemical investigation of the fungal strain *Talaromyces flavus* BYD07-13 was carried out, which led to the isolation of six new talapolyesters A–F (**1**–**4**, **14**, and **16**) and 11 known 15G256 polyesters (**5**–**13**, **15**, and **17**) (Fig. 1). Among them, **1**–**4** and **16** are characterized by being assembled with continuous two A-units, which is an unknown manner before. The structure elucidation of new talapolyesters, including the determination of the absolute configurations, is described. To the best of our knowledge, this is the first systematic investigation of the cytotoxicity of 15G256 polyesters, and a tight structure–activity relationship is proposed.

2. Results and discussion

2.1. Structure elucidation

A solid culture of *T. flavus* on cooked rice was extracted with EtOAc thoroughly. The EtOAc-soluble portion was subjected to repeated column chromatographies including HPLC to yield six new compounds (**1**–**4**, **14**, and **16**) and 11 known ones.

Talapolyester A (**1**) was isolated as a yellow oil, and the molecular formula was established by HRESIMS as $C_{22}H_{30}O_{12}$ (eight degrees of unsaturation). The IR spectrum exhibited intense and broad absorption bands at ν_{max} 3410 and 1733 cm^{-1}, indicating the presence of hydroxyl and ester carbonyl groups, respectively. The ^1H and ^{13}C NMR spectra (Table 1) combined with DEPT 135 spectrum displayed 22 carbon signals including one tetra-substituted benzene ring, three methyls, five methylenes, four methines, and four ester carbonyls. The ^1H–^1H COSY correlations between H-3 and H_a-2/$H_{a,b}$-4, between H-13 and H_a-12/H_3-14, between H-17 and $H_{a,b}$-16/H_3-18, and between H-21 and $H_{a,b}$-20/H_3-22, in

* Corresponding authors. Tel.: +86 20 85221559; e-mail addresses: tghao@jnu.edu.cn (H. Gao), tyaoxs@jnu.edu.cn (X.-S. Yao).
† These authors contributed equally to this work.

0040-4020/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tet.2014.02.060

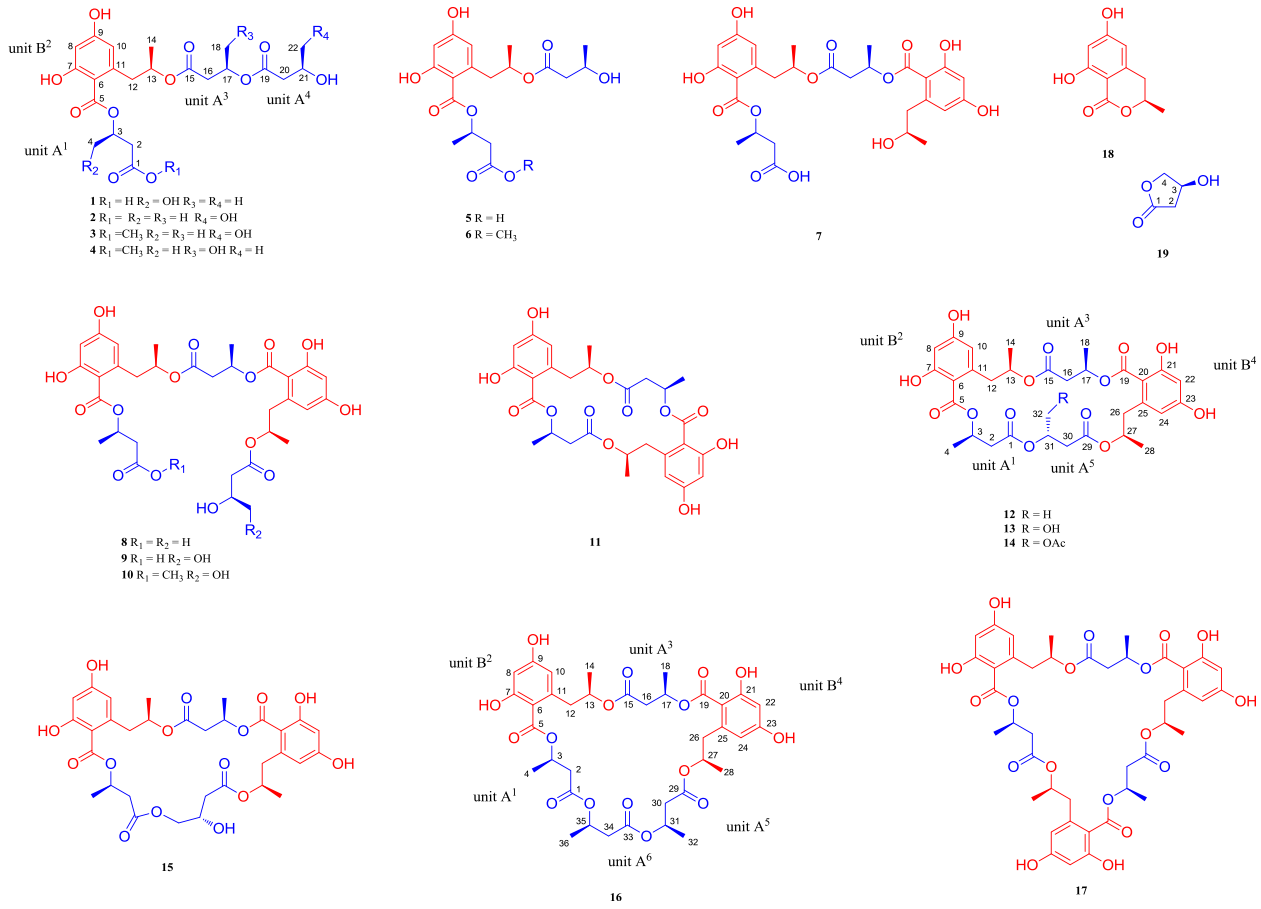

Fig. 1. Structures of compounds **1**–**19**.

combination with the HMBC correlations from $H_{a,b}$-2/H-3 to C-1, from H-8 to C-5/C-6/C-10, from $H_{a,b}$-12 to C-6/C-10/C-11/C-14, from $H_{a,b}$-16/H-17 to C-15, and from $H_{a,b}$-20/H-21 to C-19 established four units in **1** (Fig. 2). The linkage among these units was readily assigned by HMBC spectral data, which displayed correlations of H-3/$H_{a,b}$-4 to C-5, H-13 to C-15, and H-17 to C-19. Thus, the planar structure of **1** was elucidated as shown in Fig. 1. The building blocks of **1** were 2,4-dihydroxy-6-(2-hydroxypropyl)benzoic acid and 3-hydroxybutyric acid, the same as those of the known 15G256 polyesters.[1] The absolute configuration of each residue in **1** was deduced from the negative rotation of its alkaline hydrolyzates, (−)-(*R*)-6-hydroxymellein (**18**, $[\alpha]_D^{25}$ −47.6, CD (*c* 9.3×10^{-5} M, CH_3OH) λ_{max} nm ($\Delta\varepsilon$): 215 (+1.28), 232 (−8.64), 247 (+0.41), 267 (−7.26), 301 (+1.15))[1,19] and (−)-(*S*)-3-hydroxy-butyrolactone (**19**, $[\alpha]_D^{25}$ −73.4).[1,20] Moreover, the absolute configuration of **19** was confirmed by the modified Mosher's method.[21] The $\Delta\delta$ values of the (*S*)- and (*R*)-MTPA esters (**19a** and **19b**) indicated the *S* configuration for C-3. Since (*R*)-2,4-dihydroxy-6-(2-hydroxypropyl)benzoic acid is the ring-opened form of **18**, and (*R*)-3-hydroxybutyric acid is the natural precursor of **19**. Thus, the absolute stereochemistry of **1** was determined as 3*S*,13*R*,17*R*, and 21*R*.

Talapolyester B (**2**) was assigned the same molecular formula $C_{22}H_{30}O_{12}$ (eight degrees of unsaturation) as **1**. The NMR spectroscopic data suggest **2** was very similar to **1** (Table 1) except for the position of the primary hydroxyl group, which was located at C-22 for **2** instead of at C-4 based on the $^1H-^1H$ COSY correlations between H-21 and $H_{a,b}$-20/$H_{a,b}$-22, together with HMBC correlations from $H_{a,b}$-22 to C-20/C-21. The linkage among the building units was readily confirmed by HMBC correlations of H-3 to C-5, H-13 to C-15, and H-17 to C-19 (Fig. 2).

Talapolyester C (**3**) was isolated as a yellow oil. Its molecular formula was established as $C_{23}H_{32}O_{12}$ (eight degrees of unsaturation) by HRESIMS. Comparing the ^1H and ^{13}C NMR spectra of **3** with those of **2** (Table 1) made it apparent that an additional methoxy group signal was present at δ_H/δ_C: 3.60/52.3. The methoxy group was located at C-1 by the HMBC correlation from δ_H 3.60 to δ_C 172.6 (C-1) (Fig. 2). The absolute configurations of the residues in **3** were determined by alkaline hydrolysis. Thus, the absolute configuration of **3** was determined as 3*R*,13*R*,17*R*, and 21*S*. Due to a shortage of the amount of **2**, the absolute configuration of **2** was deduced as the same as **3** based on the co-occurrence of **2** and **3**.

Talapolyester D (**4**) was assigned the same molecular formula $C_{23}H_{32}O_{12}$ (eight degrees of unsaturation) as **3**. The NMR spectroscopic data suggest **4** was very similar to **3** (Table 1) except for the position of the primary hydroxyl group, which was located at C-18 for **4** instead of at C-22 based on the $^1H-^1H$ COSY correlations between H-17 and $H_{a,b}$-16/$H_{a,b}$-18, together with HMBC correlations from $H_{a,b}$-18 to C-16/C-17 (Fig. 2). The linkage among the building units was readily strengthened by HMBC correlations of H-3 to C-5, H-13 to C-15, and H-17 to C-19. The absolute configurations of the residues in **4** were deduced from its alkaline hydrolyzates. Thus, the absolute configuration of **4** was determined as 3*R*,13*R*,17*S*, and 21*R*.

Talapolyester E (**14**) was obtained as a yellow powder, possessing the molecular formula $C_{34}H_{40}O_{16}$ (15 degrees of unsaturation) as revealed from its HRESIMS data. The ^1H and ^{13}C NMR spectra (Table 2) were similar to those of the known compound 15G256α (**13**),[1] except for signals from an additional acetyl group [δ_H 2.02 (3H, s); δ_C 20.6 and 172.3]. The acetyl group was located at C-32 based on the HMBC cross-peak between $H_{a,b}$-32 and C-33. The

Table 1
^{13}C and ^{1}H NMR data for **1**–**4** in CD_3OD

No.	**1**[a]		**2**[b]		**3**[a]		**4**[a]	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
	Unit A[1]		Unit A[1]		Unit A[1]		Unit A[1]	
1	175.0		173.7		172.6		172.6	
2	39.7	2.42 (dd, 15.7, 8.0), H_a 2.50 (dd, 15.7, 4.7), H_b	41.6	2.75 (dd, 15.8, 5.6), H_a 2.82 (dd, 15.8, 4.2), H_b	41.6	2.74 (dd, 15.8, 5.4), H_a 2.79 (dd, 15.8, 5.8), H_b	41.4	2.80 (m), H_a 2.82 (m), H_b
3	67.3	4.19 (m)	70.2	5.58 (m)	70.1	5.57 (m)	70.2	5.60 (m)
4	68.8	4.08 (m), H_a 4.10 (m), H_b	20.1	1.45 (d, 6.3)	20.1	1.44 (d, 6.3)	20.2	1.45 (d, 6.3)
	Unit B[2]		Unit B[2]		Unit B[2]		Unit B[2]	
5	171.6		171.3		171.2		171.6	
6	106.0		105.9		105.8		105.8	
7	166.1		166.2		166.3		166.2	
8	102.7	6.19 (d, 2.3)	102.7	6.19 (d, 2.4)	102.7	6.19 (d, 2.5)	102.7	6.19 (d, 2.5)
9	163.5		163.5		163.6		163.5	
10	113.8	6.20 (d, 2.3)	113.9	6.20 (d, 2.4)	113.9	6.20 (d, 2.5)	113.9	6.20 (d, 2.5)
11	143.6		143.7		143.7		143.6	
12	43.4	2.89 (dd, 13.5, 9.1), H_a 3.24 (dd, 13.5, 4.3), H_b	43.6	2.86 (dd, 13.6, 9.5), H_a 3.28 (dd, 13.6, 4.1), H_b	43.7	2.85 (dd, 12.9, 9.2), H_a 3.29 (m), H_b	43.5	2.87 (dd, 13.4, 9.1), H_a 3.24 (dd, 13.4, 4.2), H_b
13	72.7	5.19 (m)	72.7	5.19 (m)	72.7	5.19 (m)	72.6	5.19 (m)
14	20.5	1.28 (d, 6.2)	20.6	1.28 (d, 6.2)	20.6	1.28 (d, 6.2)	20.5	1.28 (d, 6.2)
	Unit A[3]		Unit A[3]		Unit A[3]		Unit A[3]	
15	172.6		172.6		172.5		173.1	
16	45.1	2.27 (dd, 14.8, 6.3), H_a 2.37 (dd, 14.8, 6.9), H_b	45.1	2.27 (dd, 14.8, 6.4), H_a 2.37 (dd, 14.8, 6.9), H_b	45.1	2.27 (dd, 14.8, 6.4), H_a 2.37 (dd, 14.8, 6.9), H_b	39.4	2.43 (dd, 15.6, 7.9), H_a 2.53 (dd, 15.6, 4.8), H_b
17	65.4	4.03 (m)	65.4	4.03 (m)	65.4	4.03 (m)	67.1	4.19 (m)
18	23.0	1.06 (d, 6.2)	23.0	1.06 (d, 6.2)	23.0	1.06 (d, 6.2)	68.6	4.05 (m), H_a 4.11 (dd, 11.2, 5.7), H_b
	Unit A[4]		Unit A[4]		Unit A[4]		Unit A[4]	
19	171.8		171.6		171.6		171.7	
20	41.4	2.78 (dd, 15.9, 8.5), H_a 2.83 (dd, 15.9, 5.5), H_b	36.7	2.57 (dd, 16.1, 7.5), H_a 2.63 (dd, 16.1, 5.7), H_b	36.3	2.59 (dd, 16.1, 7.9), H_a 2.67 (dd, 16.1, 5.0), H_b	45.1	2.27 (dd, 14.8, 6.4), H_a 2.37 (dd, 14.8, 6.9), H_b
21	70.2	5.59 (m)	73.3	5.24 (m)	72.9	5.24 (m)	65.4	4.02 (m)
22	20.2	1.45 (d, 6.3)	63.6	3.60 (dd, 11.9, 5.6), H_a 3.62 (dd, 11.9, 4.5), H_b	63.5	3.58 (m), H_a 3.59 (m), H_b	23.0	1.06 (d, 6.3)
1-OCH_3					52.3	3.60 (s)	52.2	3.64 (s)

[a] Measured at 400 MHz for ^{1}H and 100 MHz for ^{13}C.
[b] Measured at 600 MHz for ^{1}H and 150 MHz for ^{13}C.

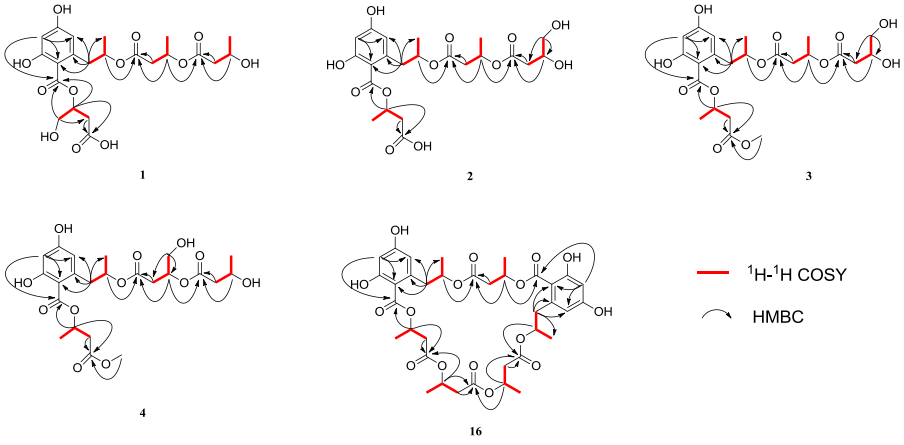

Fig. 2. Selected HMBC and ^{1}H–^{1}H COSY correlations of compounds **1**–**4** and **16**.

absolute configurations of the residues in **14** were determined by alkaline hydrolysis. Thus, the absolute configuration of **14** was determined as 3*R*,13*R*,17*R*,27*R*, and 31*S*.

Talapolyester F (**16**) was obtained as a yellow powder. The HRESIMS spectrum indicated that the molecular formula was $C_{36}H_{44}O_{16}$ (15 degrees of unsaturation). The ^{1}H and ^{13}C NMR spectra (Table 2) combined with DEPT 135 spectrum displayed 36 carbon signals including two tetra-substituted benzene rings, six methyls, six methylenes, six methines, and six ester carbonyls, which account for 14 degrees of unsaturation. The remaining one degree of unsaturation required the presence of a ring. The ^{1}H–^{1}H COSY and HSQC-TOCSY spectra (Fig. 2) showed six isolated spin-systems C-2/C-3/C-4, C-12/C-13/C-14, C-16/C-17/C-18, C-26/C-27/C-28, C-30/C-31/C-32, and C-34/C-35/C-36. Furthermore, the HMBC correlations (Fig. 2) from H-3/$H_{a,b}$-2 to C-1, from H-8 to C-5/C-6/C-10, from $H_{a,b}$-12 to C-6/C-10/C-11/C-13/C-14, from H-17/$H_{a,b}$-16 to C-15, from H-22 to C-19/C-20/C-24, from $H_{a,b}$-26 to C-20/C-24/C-25/C-27/C-28, from H-31/$H_{a,b}$-30 to C-29, and from H-35/$H_{a,b}$-34 to C-33 established six moieties in **16**. The linkage among these moieties were readily assigned by HMBC correlations from H-3 to

Please cite this article in press as: He, J.-W.; et al., Tetrahedron (2014), http://dx.doi.org/10.1016/j.tet.2014.02.060

Table 2
^{13}C and ^{1}H NMR data for **14** and **16** in CD_3OD

No.	**14**[a]		**16**[b]	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
	Unit A[1]		Unit A[1]	
1	171.3		171.5	
2	40.9	2.75 (m), H_a 2.85 (m), H_b	41.3	2.68 (dd, 16.0, 6.7), H_a 2.89 (dd, 16.0, 6.4), H_b
3	69.7	5.53 (m)	70.1	5.53 (m)
4	20.1	1.41 (d, 6.2)	20.0[c]	1.42 (d, 6.3)
	Unit B[2]		Unit B[2]	
5	170.9		171.0[d]	
6	106.7		106.1	
7	165.4		165.9	
8	102.9	6.21 (d, 2.1)	102.7	6.19 (d, 2.4)
9	163.6		163.5	
10	113.4	6.23 (d, 2.1)	113.3	6.21 (d, 2.4)
11	143.2		143.5	
12	42.5	2.79 (m), H_a 3.42 (dd, 13.1, 6.5), H_b	42.9	3.03 (m), H_a 3.20 (dd, 13.5, 8.4), H_b
13	73.6	5.00 (m)	73.8	5.10 (m)
14	19.6	1.12 (d, 6.2)	20.5	1.21 (d, 6.3)
	Unit A[3]		Unit A[3]	
15	171.1		171.1[d]	
16	41.6	2.69 (m), H_a 2.81 (m), H_b	41.8	2.62 (m), H_a 2.78 (dd, 15.4, 7.2), H_b
17	70.1	5.50 (m)	70.4	5.47 (m)
18	20.1	1.39 (d, 6.2)	19.9[c]	1.27 (d, 6.3)
	Unit B[4]		Unit B[4]	
19	171.0		171.2[d]	
20	107.3		107.4	
21	165.0		164.8	
22	102.7	6.19 (d, 2.1)	102.7	6.18 (d, 2.4)
23	163.4		163.3	
24	112.7	6.24 (d, 2.1)	112.6	6.25 (d, 2.4)
25	143.0		143.3	
26	42.0	2.92 (dd, 13.3, 7.2), H_a 3.34 (m), H_b	41.8	2.99 (m), H_a 3.09 (m), H_b
27	73.9	5.03 (m)	72.8	5.15 (m)
28	19.7	1.16 (d, 6.3)	20.3	1.20 (d, 6.2)
	Unit A[5]		Unit A[5]	
29	171.2		171.4	
30	36.7	2.64 (m), H_a 2.66 (m), H_b	41.8	2.42 (m), H_a 2.56 (m), H_b
31	70.3	5.42 (m)	68.8	5.20 (m)
32	65.4	4.04 (dd, 12.0, 5.7), H_a 4.32 (dd, 12.0, 3.4), H_b	19.8[c]	1.17 (d, 6.2)
			Unit A[6]	
33	172.3		171.2[d]	
34	20.6	2.02 (s)	41.4	2.46 (m), H_a 2.50 (m), H_b
35			69.1	5.26 (m)
36			19.9[c]	1.26 (d, 6.3)

[a] Measured at 400 MHz for ^{1}H and 100 MHz for ^{13}C.
[b] Measured at 300 MHz for ^{1}H and 75 MHz for ^{13}C.
[c] The assignment of carbons can be interchanged.
[d] The assignment of carbons can be interchanged.

C-5, H-13 to C-15, H-17 to C-19, H-27 to C-29, H-31 to C-33, and H-35 to C-1. Thus, a planar structure was assigned to **16**. The absolute configuration of the (*R*)-2,4-dihydroxy-6-(2-hydroxypropyl)benzoic acid residue in **16** was determined by alkaline hydrolysis. Moreover, the absolute configuration of 3-hydroxybutyric acid residue was deduced as *R* from the biosynthetic perspective. Therefore, the absolute configuration of **16** was determined to be 3*R*,13*R*,17*R*,27*R*,31*R*, and 35*R*.

On the basis of the HRESIMS, NMR spectroscopic data, specific rotations, CD data, as well as the modified Mosher's method, the 13 known compounds were determined to be 15G256ν (**5**),[1] 15G256ν-me (**6**),[1] 15G256π (**7**),[1] 15G256β-2 (**8**),[1] 15G256α-2 (**9**),[1] 15G256α-2-me (**10**),[1] 15G256ι (**11**),[1] 15G256β (**12**),[1] 15G256α (**13**),[1] 15G256α-1 (**15**),[1] 15G256ω (**17**),[1] (*R*)-6-hydroxymellein (**18**),[1,19] and (*S*)-3-hydroxy-butyrolactone (**19**).[1,20]

To verify that talapolyesters A–F (**1**–**4**, **14**, and **16**) are the authentic natural products, a portion of the moderate extraction was subjected to UPLC–HRESIMS analysis. This suggests that **1**–**4**, **14**, and **16** are unlikely to be isolation artifacts because they were detected.

Based on the biosynthetic pathway proposed by Schlingman's,[1] we suggest a plausible biogenesis of **1**–**4** and **16**. Compounds **1**–**4** may originate from **5** via attaching an A-unit, while **8** could undergo ring closure following receive a final A-unit to form **16**. For those known polyesters, based on the same precursors **5** and **8**, compounds **7**, **11**, **12**, and **17** were generated by adding a B-unit or direct ring closure.[1] Compounds **1**–**4** and **16** are characterized by being assembled with continuous two A-units, which is an unknown manner before.

2.2. Cytotoxic bioassay

It was reported that compounds **12**, **13**, and **15** showed toxicity against mouse peritoneal macrophage.[4] Herein, compounds **1**–**19** were evaluated by MTT method[22,23] for their cytotoxic activities against five tumor cell lines, HL-60, SMMC-7721, A-549, MCF-7, and SW480, with cisplatin and paclitaxel as the positive controls. As shown in Table 3, all macrocyclic polyesters (**11**–**17**) exhibited cytotoxicity against the tested tumor cell lines, while linear polyesters (**1**–**10**) and two organic acid lactones (**18** and **19**) were inactive (IC_{50}>40 μM) as compared to cisplatin. This suggests that a macrocyclic structure is required for cytotoxicity. Among the cyclic compounds, **12** and **17** exhibited significant activity against MCF-7 cell line with the IC_{50} of 3.27 and 4.32 μM, respectively. This is the first systematic investigation on cytotoxicity of 15G256 polyesters, and a tight structure–activity relationship is proposed.

Table 3
Cytotoxicity of compounds **1**–**19** (IC50, μM)

Compounds	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	>40	>40	>40	>40	>40
2	>40	>40	>40	>40	>40
3	>40	>40	>40	>40	>40
4	>40	>40	>40	>40	>40
5	>40	>40	>40	>40	>40
6	>40	>40	>40	>40	>40
7	>40	>40	>40	>40	>40
8	>40	>40	>40	>40	>40
9	>40	>40	>40	>40	>40
10	>40	>40	>40	>40	>40
11	17.95	38.70	18.96	12.26	>40
12	14.74	15.45	12.06	3.27	15.57
13	17.55	29.10	23.84	18.53	>40
14	14.81	18.39	17.66	14.59	26.62
15	22.12	>40	36.01	19.67	>40
16	13.62	15.74	11.09	15.96	15.54
17	12.46	13.08	10.61	4.32	12.52
18	>40	>40	>40	>40	>40
19	>40	>40	>40	>40	>40
Cisplatin[a]	1.32	6.24	11.83	15.17	12.95
Paclitaxel[a]	<0.008	<0.008	<0.008	<0.008	<0.008

[a] Positive control.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a JASCO P-1020 digital polarimeter at room temperature. IR spectra were obtained on a JASCO FT/IR-480 plus Fourier transform infrared spectrometer. CD spectrum was recorded in CH_3OH using a JASCO J-810

spectrophotometer at room temperature. HRESIMS spectra were obtained on Waters Synapt G2 TOF mass spectrometer. NMR data were recorded on Bruker AV 300, 400, and 600 using solvent signals (CD_3OD: δ_H 3.30/δ_C 49.0) as references. Column chromatography was carried out on silica gel (200–300 mesh) (Qingdao Haiyang Chemical Group Corporation, Qingdao, China), Sephadex LH-20 (Pharmacia), and ODS (60–80 μm, YMC), respectively. Preparative HPLC was performed on Shimadzu LC-6AD system equipped a diode array detector, using an RP-18 column (250×21.2 mm, 5 μm, Gemini, Phenomenex; detector set at 220 nm and 254 nm) with a flow rate of 8.0 mL/min. All solvents used in column chromatography were of analytical grade (Tianjin Damao Chemical Plant, Tianjin, China).

3.2. Fungal material

The strain BYD07-13 was isolated from a soil sample, collected from Baiyangdian, Hebei province of China. The fungal strain was identified as *T. flavus* based on the morphological characters and gene sequence analyses. A blast search of its ITS (KF917583), calmodulin (KF917585), and β-tubulin sequence (KF917584) showed that these sequences matched well with the corresponding genes (JN602366.1, AY678609.1, and AY766252.1) from *T. flavus* with sequence identify of 99%, 90%, and 95%, respectively. The detailed identification method for gene sequencing analyses is described below.

The fresh fungal mycelia (about 50 mg) were obtained by centrifuging 1 mL of fresh culture in a 1.5 mL micro-centrifuge tube. 100 μL of preheated (60 °C) 2xCTAB extraction buffer (2% CTAB (w/v), 100 mM Tris–HCl, 1.4 M NaCl, 20 mM EDTA, pH 8.0) and 0.2 g sterilized quartz sand (Sangon Biotech Co., Ltd, Shanghai, China) were added, and fungal mycelia were ground using a glass pestle. 500 μL of preheated (60 °C) 2xCTAB extraction buffer was added, and incubated at 60 °C for 30 min with occasional gentle swirling. After that, 500 μL of phenol–chloroform (1:1, v/v) was added and mixed thoroughly to form an emulsion. The mixture was centrifuged at 10,000 rpm for 10 min at room temperature. The aqueous phase (500 μL) was removed into a fresh 1.5 mL tube and extracted with 500 μL chloroform. The mixture was centrifuged at 10,000 rpm for 10 min at room temperature and the aqueous phase (400 μL) was transferred into a new 1.5 mL tube. Isopropanol (400 μL) was added and the genomic DNA in the aqueous phase was precipitated by centrifuging at 10,000 rpm for 10 min. The DNA pellet was washed with 70% ethanol twice, dried, and suspended in 50 μL H_2O. The primer pairs ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′), Bt2a (5′-GGTAACCAAATCGTGCTTTC-3′) and Bt2b (5′-ACCCTCAGTGTAGTGACCCTTGGC-3′), CF1 (5′-AGGCGGGAYTCTYTGACYGA-3′) and CF4 (5′-TTTYGCATCATRAGYTGGAC-3′), were used for amplification of ITS fragment, β-tubulin gene, and calmodulin gene, respectively. Amplification of ITS fragment was performed using TaKaRa PCR Amplification Kit (Takara, Dalian, China), while the β-tubulin and calmodulin genes were amplified using 2×EasyTaq PCR SuperMix (TransGen Biotech, Beijing, China). After PCR, 4 μL of reaction mixture was loaded onto an agarose gel (1% agarose) and subjected to electrophoresis. After the bands due to the PCR products (approximate size 550 bp for partial ITS, 500 bp for partial β-tubulin gene, and 1050 bp for partial calmodulin gene) were confirmed, the PCR products were then submitted for sequencing (Sangon Biotech, Shanghai, China) with the corresponding primers. The sequences data have been submitted to GenBank.

The fungal strain was cultured on slants of potato dextrose agar (PDA) at 25 °C for 5 days. Agar plugs were used to inoculate four Erlenmeyer flasks (250 mL), each containing 100 mL of potato dextrose broth (PDB). Four flasks of the inoculated media were incubated at 25 °C on a rotary shaker at 200 rpm for 5 days to prepare the seed culture. Fermentation was carried out in 22 Erlenmeyer flasks (500 mL), each containing 70 g of rice. Distilled H_2O (105 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 120 °C for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at room temperature for 55 days.

3.3. Extraction and isolation

The fermented material was extracted three times with EtOAc, and the organic solvent was evaporated to dryness under vacuum to afford the crude extract (46.2 g), which was dissolved in 90% v/v aqueous methanol (500 mL) and partitioned against an equal volume of cyclohexane (three times). The aqueous methanol layer was evaporated to dryness under reduced pressure to give an aqueous methanol extract (w, 28.1 g, the polyester-enriched fraction), which was subjected to ODS column chromatography (CC) using a CH_3OH–H_2O gradient elution to give four fractions (w1 to w4). Fraction w4 (3.74 g) was separated by silica gel CC eluting with cyclohexane–EtOAc (90:10, 80:20, 70:30, 60:40, 0:100, v/v) to afford five subfractions. Subfraction w4d (1.2 g) was separated by Sephadex LH-20 CC with elution by CH_3OH, and further purified by RP-HPLC using 63% CH_3OH in H_2O to afford **15** (134.2 mg, t_R=35.9 min) and **13** (79.2 mg, t_R=42.8 min). Fractions w2 (4.96 g) and w3 (15.7 g) were combined together and further purified using a silica gel column with a CH_2Cl_2–CH_3OH gradient elution to give seven subfractions (w2a to w2g). Subfraction w2d (1.73 g) was further separated by a silica gel column with elution by cyclohexane–EtOAc (90:10, 85:15, 80:20, 70:30, 60:40, 0:100, v/v) to afford seven subfractions. Compounds **11** (6.5 mg, t_R=23.2 min) and **12** (212 mg, t_R=31.8 min) were obtained by RP-HPLC using 68% CH_3OH in H_2O from subfraction w2d4 (545 mg). Subfraction w2d6 (779 mg) was separated by RP-HPLC using 63% CH_3OH in H_2O to afford **6** (40.2 mg, t_R=16.8 min), **15** (9.4 mg, t_R=35.9 min), **13** (5.6 mg, t_R=42.8 min), **14** (35.4 mg, t_R=67.4 min), and **16** (19.3 mg, t_R=101.5 min). Subfraction w2e (6.12 g) was further separated by a silica gel column eluting with $CHCl_3$–CH_3OH (20:1, 10:1, 5:1, 3:1, 0:100, v/v) to afford eight subfractions. Subfraction w2e3 (1.95 g) was further separated a silica gel column eluting with $CHCl_3$–CH_3OH (30:1, 20:1, 10:1, 5:1, 0:100 v/v) to afford eight subfractions. Subfraction w2e3a (529 mg) was purified by RP-HPLC using 58% CH_3OH in H_2O to afford **9** (22.5 mg, t_R=13.2 min), **7** (7.9 mg, t_R=14.8 min), **8** (6.9 mg, t_R=17.4 min), and **10** (51.5 mg, t_R=20.4 min). Compound **17** (20.6 mg, t_R=35.5 min) was obtained by RP-HPLC using 70% CH_3OH in H_2O from subfraction w2e3b (456 mg). Subfraction w2e3f (448 mg) was purified by RP-HPLC using 23% CH_3CN in H_2O with 0.05% formic acid to afford **2** (2.6 mg, t_R=21.4 min), **1** (12.6 mg, t_R=24.5 min), **5** (89.1 mg, t_R=37.1 min), **3** (6.4 mg, t_R=44.6 min), and **4** (18.9 mg, t_R=51.2 min).

*3.3.1. Talapolyester A (**1**).* Yellow oil; $[\alpha]_D^{25}$ −45.4 (*c* 1.0, CH_3OH); IR (KBr) ν_{max} cm^{-1}: 3410, 2979, 2931, 1733, 1648, 1619, 1314, 1261, 1049; for ^1H and ^{13}C NMR data, see Table 1; HRESIMS (positive) $[M+H]^+$ *m/z* 487.1813 (calcd for $C_{22}H_{31}O_{12}$, 487.1816).

*3.3.2. Talapolyester B (**2**).* Yellow oil; $[\alpha]_D^{25}$ −49.7 (*c* 1.0, CH_3OH); IR (KBr) ν_{max} cm^{-1}: 3411, 2979, 2931, 1733, 1649, 1624, 1311, 1261, 1049; for ^1H and ^{13}C NMR data, see Table 1; HRESIMS (positive) $[M+Na]^+$ *m/z* 509.1636 (calcd for $C_{22}H_{30}O_{12}Na$, 509.1635).

*3.3.3. Talapolyester C (**3**).* Yellow oil; $[\alpha]_D^{25}$ −42.2 (*c* 1.0, CH_3OH); IR (KBr) ν_{max} cm^{-1}: 3418, 2979, 2935, 1735, 1649, 1618, 1314, 1261, 1049; for ^1H and ^{13}C NMR data, see Table 1; HRESIMS

(positive) $[M+Na]^+$ m/z 523.1796 (calcd for $C_{23}H_{32}O_{12}Na$, 523.1791).

*3.3.4. Talapolyester D (**4**).* Yellow oil; $[\alpha]_D^{25}$ −39.4 (*c* 1.0, CH_3OH); IR (KBr) ν_{max} cm^{-1}: 3418, 2979, 2935, 1735, 1649, 1618, 1314, 1261, 1049; for ^1H and ^{13}C NMR data, see Table 1; HRESIMS (positive) $[M+Na]^+$ m/z 523.1795 (calcd for $C_{23}H_{32}O_{12}Na$, 523.1791).

*3.3.5. Talapolyester E (**14**).* Yellow powder; $[\alpha]_D^{25}$ −20.7 (*c* 1.0, CH_3OH); IR (KBr) ν_{max} cm^{-1}: 3411, 2984, 2935, 1734, 1650, 1619, 1314, 1260, 1054; for ^1H and ^{13}C NMR data, see Table 2; HRESIMS (positive) $[M+H]^+$ m/z 705.2393 (calcd for $C_{34}H_{41}O_{16}$, 705.2395).

*3.3.6. Talapolyester F (**16**).* Yellow powder; $[\alpha]_D^{25}$ −16.2 (*c* 1.0, CH_3OH); IR (KBr) ν_{max} cm^{-1}: 3412, 2984, 2931, 1734, 1650, 1623, 1314, 1261, 1049; for ^1H and ^{13}C NMR data, see Table 2; HRESIMS (positive) $[M+H]^+$ m/z 733.2714 (calcd for $C_{36}H_{45}O_{16}$, 733.2708).

3.4. Absolution configurations

Separate solutions of **1** (5 mg), **3** (5 mg), **4** (5 mg), **14** (10 mg), and **16** (10 mg) in 1 N NaOH were stirred for 24 h at room temperature, and the reactions were quenched by acidification with 1 N HCl solution. The reaction mixture of **1** was purified by RP-HPLC to afford **18** (53% CH_3OH in H_2O, 1.8 mg, t_R=17.8 min, white powder, $[\alpha]_D^{25}$ −47.6 (*c* 1.0, CH_3OH)), and the residue was refluxed with methylbenzene for 10 h at 130 °C. Then, it was subjected to short-path distillation (130 °C, 0.1 mm)[24] to give **19** (0.4 mg, colorless oil, $[\alpha]_D^{25}$ −73.4 (*c* 0.1, CH_3OH)). Similarly, **3**, **4**, **14**, and **16** were processed separately in the same way as **1**. However, the reaction mixture of **16** only afforded **18**. Moreover, we have also obtained **18** and **19** from the polyester-enriched fraction (w, 300 mg) in the same manner as **1**.

3.5. Preparation of (*S*)- and (*R*)-MTPA esters of 19 (19a and 19b)[21]

A solution of **19** (0.5 mg) in pyridine-d_5 (0.5 mL) was treated with (*S*)-MTPA chloride (12 μL) under an atmosphere of nitrogen in an NMR tube. The mixture was stirred at room temperature for 12 h to obtain the (*R*)-MTPA ester (**19b**). The same procedure was used to prepare the (*S*)-MTPA ester (**19a**) with (*R*)-MTPA chloride.

The $\Delta\delta$ values ($\Delta\delta_{H\text{-}2}$: −0.14643 and −0.03152; $\Delta\delta_{H\text{-}4}$: +0.14524 and +0.04753) of the (*S*)- and (*R*)-MTPA esters (**19a** and **19b**) indicated the *S* configuration for C-3 in **19**.

3.6. MTT assay[22,23]

Five human cancer lines, human myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7, and colon cancer SW480, were used in the cytotoxicity assay. All the cells were cultured in DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA), in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTT method in 96-well microplates with cisplatin and paclitaxel (Sigma, USA) as the positive controls. Cell viability after 48 h after treatment was examined and the cell growth curve was plotted. The inhibition rate was calculated and plotted versus test concentrations to afford the IC_{50}.

Acknowledgements

The manuscript adhering to correct scientific English was provided by Dr. L. J. Sparvero at the University of Pittsburgh. This work was supported by grants from Technology of China (No. 2012ZX09301002003), by the Natural Science Foundation of China (No. 81373306), by Guangdong Natural Science Funds for Distinguished Young Scholar (No. S2013050014287), by the program for New Century Excellent Talents in University (No. NCET-10-0120), and Fok Ying Tung Education Foundation (No. 121039) from the Ministry of Education of China.

Supplementary data

^1H, ^{13}C, and 2D NMR spectra of new compounds **1**–**4**, **14**, and **16**. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2014.02.060.

References and notes

1. Schlingmann, G.; Milne, L.; Carter, G. T. *Tetrahedron* **2002**, *58*, 6825–6835.
2. Abbanat, D.; Leighton, M.; Maiese, W.; Jones, E. B. G.; Pearce, C.; Greenstein, M. *J. Antibiot.* **1998**, *51*, 296–302.
3. Ito, M.; Maruhashi, M.; Sakai, N.; Mizoue, K.; Hanada, K. *J. Antibiot.* **1992**, *45*, 1559–1565.
4. Breinholt, J.; Jensen, G. W.; Nielsen, R. I.; Olsen, C. E.; Frisvad, J. C. *J. Antibiot.* **1993**, *46*, 1101–1108.
5. Roy, K.; Chatterjee, S.; Deshmukh, S. K.; Vijayakumar, E. K. S.; Ganguli, B. N.; Fehlhaber, H. W. *J. Antibiot.* **1996**, *49*, 1186–1187.
6. Zhou, Z. Y.; Liu, R.; Jiang, M. Y.; Zhang, L.; Niu, Y.; Zhu, Y. C.; Dong, Z. J.; Liu, J. K. *Chem. Pharm. Bull.* **2009**, *57*, 975–978.
7. Arai, M.; Tomoda, H.; Okuda, T.; Wang, H. Y.; Tabata, N.; Masuma, R.; Yamaguchi, Y.; Ōmura, S. *J. Antibiot.* **2002**, *55*, 172–180.
8. Wang, H. Y.; Arai, M.; Tomoda, H.; Ōmura, S. *Chin. J. Antibiot.* **2001**, *26*, 89–93.
9. Kondo, H.; Kurama, M.; Nakajima, S.; Osada, K.; Ookura, A.; Suda, H. Jpn. Kokai Tokkyo Koho JP 05032658, 1993.
10. Silber, J.; Ohlendorf, B.; Labes, A.; Erhard, A.; Imhoff, J. F. *Mar. Drugs* **2013**, *11*, 3309–3323.
11. Sultan, S.; Shah, S. A. A.; Sun, L.; Ramasami, K.; Cole, A.; Blunt, J.; Munro, H. G. M.; Faizal, W. J. F. *Int. J. Pharm. Sci.* **2011**, *3*, 7–9.
12. Navarro, I.; Basset, J. F.; Hebbe, S.; Major, S. M.; Werner, T.; Howsham, C.; Bräckow, J.; Barrett, A. G. M. *J. Am. Chem. Soc.* **2008**, *130*, 10293–10298.
13. Ye, F.; Chen, G. D.; He, J. W.; Li, X. X.; Sun, X.; Guo, L. D.; Li, Y.; Gao, H. *Tetrahedron Lett.* **2013**, *54*, 4551–4554.
14. Chen, G. D.; Chen, Y.; Gao, H.; Shen, L. Q.; Wu, Y.; Li, X. X.; Li, Y.; Guo, L. D.; Cen, Y. Z.; Yao, X. S. *J. Nat. Prod.* **2013**, *76*, 702–709.
15. Zheng, Q. C.; Chen, G. D.; Kong, M. Z.; Li, G. Q.; Cui, J. Y.; Li, X. X.; Wu, Z. Y.; Guo, L. D.; Cen, Y. Z.; Zheng, Y. Z.; Gao, H. *Steroids* **2013**, *78*, 896–901.
16. He, J. W.; Chen, G. D.; Gao, H.; Yang, F.; Li, X. X.; Peng, T.; Guo, L. D.; Yao, X. S. *Fitoterapia* **2012**, *83*, 1087–1091.
17. Chen, G. D.; Li, Y. J.; Gao, H.; Chen, Y.; Li, X. X.; Li, J.; Guo, L. D.; Cen, Y. Z.; Yao, X. S. *Planta Med.* **2012**, *78*, 1683–1689.
18. Yang, F.; Chen, G. D.; Gao, H.; Li, X. X.; Wu, Y.; Guo, L. D.; Yao, X. S. *J. Asian Nat. Prod. Res.* **2012**, *14*, 1059–1063.
19. Schlingmann, G.; Roll, D. M. *Chirality* **2005**, *17*, S48–S51.
20. Řezanka, T.; Dvořáková, R.; Hanuš, L. O.; Dembitsky, V. M. *Eur. J. Org. Chem.* **2004**, *35*, 995–1001.
21. Liu, M. L.; Duan, Y. H.; Zhang, J. B.; Yu, Y.; Dai, Y.; Yao, X. S. *Tetrahedron* **2013**, *69*, 6574–6578.
22. Mosmman, T. *J. Immunol. Methods* **1983**, *65*, 55–63.
23. Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L. *Cancer Res.* **1988**, *48*, 589–601.
24. Moore, R. E.; Blackman, A. J.; Cheuk, C. E.; Mynderse, J. S. *J. Org. Chem.* **1984**, *49*, 2484–2489.