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BRIEF COMMUNICATION

New phenolic bisabolane sesquiterpenoid derivatives with cytotoxicity from *Aspergillus tennesseensis*

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Abstract

Three new bisabolane sesquiterpenoid esters, aspertenols A–B (**1–3**), and six known compounds (**4–9**) were isolated from the fungus *Aspergillus tennesseensis*. The structures of new compounds were elucidated by extensive spectroscopic analysis. The cytotoxicities of **1–9** against A549, K562, and ASPC cell lines were tested by using the CCK8 method. Compounds **1**, **3**, **4**, **6**, **7**, and **9** showed inhibition on K562 cell line with IC_{50} values in the range from 16.6 to 72.7 μM. Compounds **1**, **4**, and **9** showed moderate inhibitory activity against A549 with IC_{50} of 43.5, 70.2, and 61.1 μM, respectively.

Bisalolane-type sesquiterpenoids are a very important family of natural products with various bioactivities, such as cytotoxicity [1], antibacterial [2], and antioxidant [3]. Several phenolic bisabolane-type sesquiterpenes have been isolated from different organisms, such as *Pseudopterogorgia rigida* [4], *Didiscus aceratus* [5], *Myrmekioderma styx* [6], *Verticillium tenerum* [7], *Glonium* sp. [8], *Penicillium expansum* [9], *Penicillium aculeatum* [10], and *Aspergillus sydowii* [3].

Fungi of genus *Aspergillus* are known to produce a wide array of bioactive secondary metabolites. Our previous study of searching for bioactive metabolites from *Aspergillus tennesseensis*, a strain isolated from the surface of an unidentified plant leaf, yielded ten prenylated indole alkaloids, including three new natural product hybrids with unprecedented chemical skeletons [11, 12]. In our continuing study on this fungus, three new polyphenols, aspertenols A–B (**1–3**) and six known compounds (**4–9**) were isolated and identified. Here, we reported the isolation, structural elucidation, and cytotoxicity evaluation of compounds **1–9**.

This fungus was fermented on rice and extracted with ethyl acetate to afford the organic solvent extract. The obtained extract was subjected to chromatographic separation using silica gel, ODS, Sephadex LH-20, and preparative HPLC to yield nine compounds (Fig. 1), including three new bisabolane sesquiterpenoid esters aspertenols A–C (**1–3**), as well as six known compounds that identified as peniciaculin B (**4**) [10], hydroxysydonic acid (**5**) [13], sterigmatocystin (**6**) [14], 5-methoxysterigmatocystin (**7**) [15], methyl 2-hydroxy-4-(3-hydroxy-5-methylphenoxy)-6-methylbenzoate (**8**) [16], diorcinol D (**9**) [17]. The structures of known compounds (**4–9**) were determined by comparison of their spectroscopic data with the literature data.

Aspertenol A (**1**) was isolated as colorless oil. The molecular formula was determined as $C_{30}H_{36}O_7$ on the basis of HRESIMS with the $[M+Na]^+$ peak at *m/z* 531.2356, indicating 13 degrees of unsaturation. Analysis of its ^1H, ^{13}C, and HSQC NMR data (Table 1) revealed the presence of five methyl, four methylene (an oxygenated), nine methine (eight olefinic), eleven quaternary carbons (nine substituted benzenoid carbons, an ester carbonyl, and an oxygenated). Its 2D NMR showed that **1** possessed a

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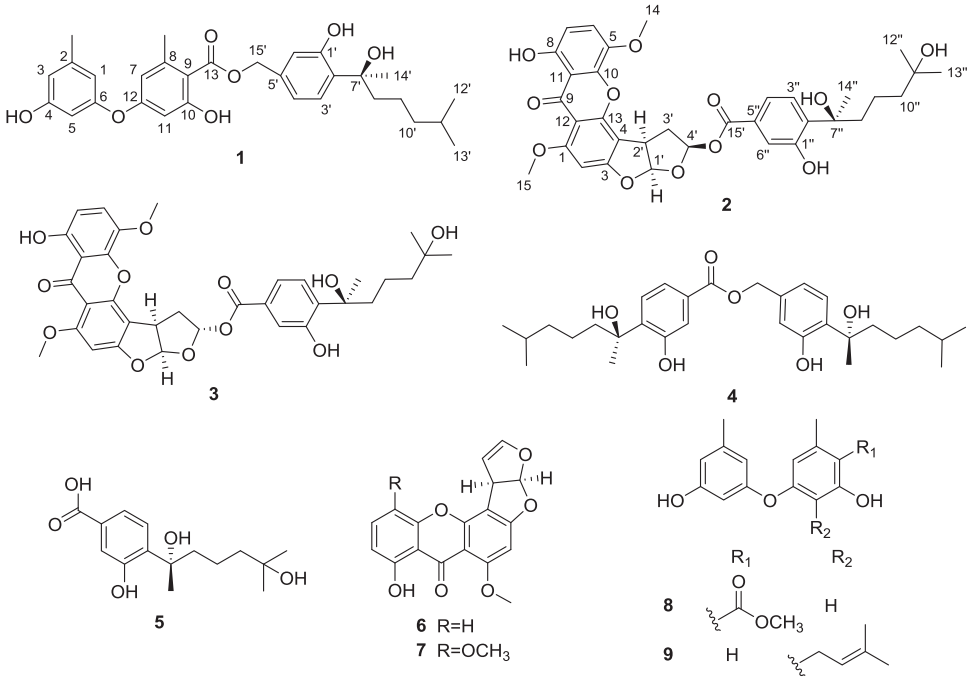

Fig. 1 Structures of compounds **1–9**

diphenyl ether substructure, similar with those of methyl 2-hydroxy-4-(3-hydroxy-5-methylphenoxy)-6-methylbenzoate (**8**), except for the absence of a methoxyl group, which was verified by the key HMBC correlations (Fig. 2) from H-1 (δ_H 6.45) to C-3 (δ_C 112.7) and C-5 (δ_C 105.1), CH_3-2 (δ_H 2.28) to C-1 (δ_C 113.7), C-2 (δ_C 141.4) and C-3, H-3 (δ_H 6.48) to C-5, H-5 (δ_H 6.35) to C-6 (δ_C 156.4), H-7 (δ_H 6.34) to C-9 (δ_C 107.1) and C-11 (δ_C 103.4), CH_3-8 (δ_H 2.51) to C-8 (δ_C 144.0) and C-9, 10-OH (δ_H 11.65) to C-9, C-10 (δ_C 165.4) and C-11 (δ_C 103.4), H-11 (δ_H 6.33) to C-9 and C-12 (δ_C 162.5). Further analysis of the remaining chemical shift in **1**, confirmed a bisabolane sesquiterpenoid substructure, similar to that of hydroxysydonic acid (**5**), except for the presence of an oxygenated methylene and a methine, and the absence of an ester carbonyl and an oxygenated quaternary carbon, which was further determined by ^{1}H–^{1}H COSY correlations (Fig. 2) of H-3′/H-4′, H_2-8′/H_2-9′/H_2-10′/H-11′/H_3-12′, H-11′/H_3-13′, and the key HMBC correlations (Fig. 2) from H-3′ (δ_H 7.00) to C-1′ (δ_C 156.8), C-5′ (δ_C 136.2) and C-7′ (δ_C 79.1), H-4′ (δ_H 6.86) to C-2′ (δ_C 129.8), C-6′ (δ_C 117.5) and C-15′ (δ_C 66.7), H_2-8′ (δ_H 1.79, 1.89) to C-2′ and C-7′, H-6′ (δ_H 6.91) to C-4′ (δ_C 119.3) and C-15′, H_3-14′ (δ_H 1.65) to C-2′ and C-7′. The linkage between two substructures via an ester carbonyl group (δ_C 171.5) was determined by HMBC correlations from CH_3-8 (δ_H 2.51) to C-13 (δ_C 171.5) and H-15′ (δ_H 5.31) to C-13. Thus, the planar structure of **1** was confirmed. The CD spectrum (Figure S1) of **1** showed two positive Cotton effect around 225 and 282 nm, similar to that of *S*-(+)-sydonol [8] and *S*-(+)-curcutetraol [18]. Thus, the absolute configuration at C-7′ of **1** was assigned as 7′*S*.

Aspertenol B (**2**) was obtained as yellow powder with a molecular formula of $C_{34}H_{36}O_{12}$, as established by HRESIMS data at *m/z* $[M+H]^+$ 637.2284. The ^{1}H and ^{13}C NMR signals in bisabolane sesquiterpenoid moiety of **2** were similar to those of compound **5**. Further detailed analysis of 1D NMR (Table 1) revealed that the remaining chemical shift in **2** showed similarity with xanthones 5-methoxysterigmatocystin (**7**), except for the presence of a methylene (δ_H 2.64, 2.82, δ_C 36.9) and an oxygenated methine (δ_H 6.73, δ_C 99.4), and the lack of two olefinic methines. Thus, we inferred that this unit of **2** was from a 3′,4′-dihydro-5-methoxysterigmatocystin residue, which was confirmed by the ^{1}H–^{1}H COSY correlations (Fig. 2) between H-6/H-7, H-1′/H-2′/H_2-3′/H-4′, along with the key HMBC correlations (Fig. 2) from H-2 (δ_H 6.47) to C-3 (δ_C 165.2), C-4 (δ_C 107.2), C-9 (δ_C181.5), C-12 (δ_C 106.1), and C-13 (δ_C 154.1), H-6 (δ_H 7.17) to C-8 (δ_C 155.1) and C-10 (δ_C 144.7), H-7 (δ_H 6.67) to C-5 (δ_C 139.6), C-9 and C-11 (δ_C 109.7), H_3-14 (δ_H 3.91) to C-5, H_3-15 (δ_H 4.01) to C-1 (δ_C 163.9) and C-2 (δ_C 91.0), H-1′ (δ_H 6.62) to C-3, C-4 and C-4′ (δ_C 99.4), H-2′ (δ_H 4.39) to C-3 and C-13, H-3′ (δ_H 2.64, 2.82) to C-4 and C-1′ (δ_C 113.9), H-4′ (δ_H 6.73) to C-2′ (δ_C 42.7). The connection between the two substructures of **2** was verified by HMBC correlations from H-4′ (δ_H 6.73) to C-15″ (δ_C 164.8). The relative configurations of **2** was established by the NOE correlations (Fig. 2) of H-1′ (δ_H 6.62) with H-2′ (δ_H 4.39), H-2′ with H-3′α (δ_H 2.64), H-3′α with H-4′ (δ_H 6.73), which indicated that H-1′, H-2′, H-3′α, and H-4′ were on the same side and H-3′β was on the opposite side. From a biosynthetic perspective, it seemed reasonable to assume that compound **2** possessed the same

Table 1 ^{1}H and ^{13}C NMR data for compounds **1–3**[a]

	1			δc	**2**	**3**[a]	δ_H (*J*in Hz)
	δc	δ_H (*J* in Hz)			δ_H (*J* in Hz)	δc	
1	113.7	6.45 s	1	163.9		163.9	
2	141.4		2	91.0	6.47 s	91.0	6.43 s
2-CH_3	21.6	2.28 s	3	165.2		165.0	
3	112.7	6.48 s	4	107.2		106.9	
4	156.1		5	139.6		139.6	
5	105.1	6.35 s	6	120.2	7.17 d(8.9)	120.3	7.19 d(9.0)
6	156.4		7	109.6	6.67 d(8.9)	109.7	6.70 d(8.9)
7	113.2	6.34 s	8	155.1		155.2	
8	144.0		9	181.5		181.5	
8-CH_3	24.8	2.51 s	10	144.7		144.7	
9	107.1		11	109.7		109.7	
10	165.4		12	106.1		106.4	
11	103.4	6.33 s	13	154.1		154.5	
12	162.5		14	57.8	3.91 s	57.7	3.92 s
13	171.5		15	56.9	4.01 s	57.0	4.01 s
1′	156.8		1′	113.9	6.62 d(5.9)	112.9	6.60 d(5.9)
2′	129.8		2′	42.7	4.39 dd(8.8,6.0)	42.8	4.45 dt(10.0,5.2)
3′	126.6	7.00 d(8.0)	3′	36.9	2.64 m	37.0	2.67 m
4′	119.3	6.86 d(8.0)			2.82 d(14.0)		2.80 m
5′	136.2		4′	99.4	6.73 o[b]	99.2	6.63 dd(5.3,3.4)
6′	117.5	6.91 s	1″	156.2		156.4	
7′	79.1		2″	135.4		135.2	
8′	43.0	1.79 m[c]	3″	126.0	6.82 d(8.2)	126.5	7.07 d(8.0)
		1.89 m	4″	120.4	6.90 dd(8.1,1.8)	121.0	7.49 o
9′	21.8	1.27 m	5″	129.5		129.8	
10′	39.1	1.16 m	6″	118.9	6.72 o	119.1	7.50 o
11′	27.9	1.50 m	7″	78.7		79.3	
12′	22.7	0.83 m	8″	42.6	1.79 m	43.0	1.87 m
13′	22.7	0.83 m			1.90 m		1.99 m
14′	29.2	1.65 s	9″	18.6	1.38 m	18.8	1.44 m
15′	66.7	5.31 s	10″	43.3	1.44 m	43.3	1.47 m
10-OH		11.65 s	11″	71.3		71.4	
1′-OH		9.22 s	12″	29.5	1.16 s	29.6	1.19 s
			13″	29.7	1.16 s	29.7	1.19 s
			14″	29.1	1.57 s	29.6	1.68 s
			15″	164.8		164.9	
			8-OH		12.60 s		12.58 s
			7″-OH		9.29 s		9.57 s

[a] Recorded for ^{1}H NMR at 500 MHz, for ^{13}C NMR at 125 MHz in $CDCl_3$, δ_H in ppm, *J* in Hz

[b] "o" signals overlapped with others

[c] 'm' means multiplet with other signals

R absolute configuration at C-1′ and C-2′ as that of **6** and **7**, the *S* absolute configuration at C-7″ that is identical with that in **1** and **5**. Thus, the absolute configuration of **2** was proposed to be 1′*R*, 2′*R*, 4′*R*, 7″*S*.

Aspertenol C (**3**) was obtained as yellow powder with the molecular formula $C_{34}H_{36}O_{12}$. Careful analysis of ^{1}H and ^{13}C NMR spectra (Table 1), particularly ^{1}H-^{1}H COSY, HSQC, and HMBC (Fig. 2) revealed a same planar structure

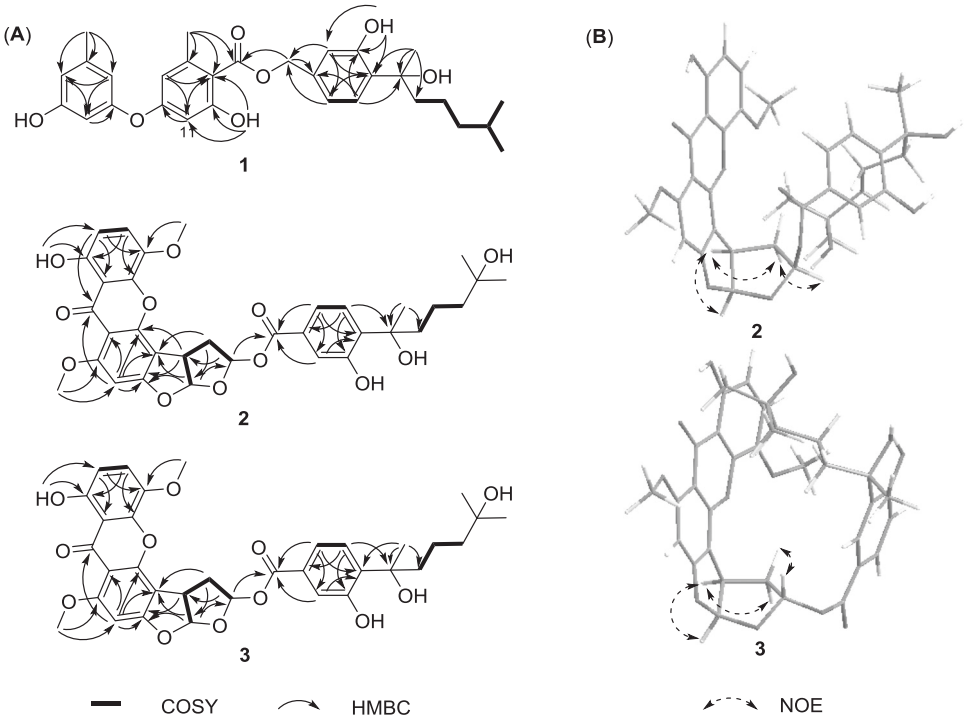

Fig. 2 **a** Key 1H-1H COSY and HMBC correlations of **1–3**. **b** Key NOE correlations of **2** and **3** (Color figure online)

Table 2 Cytotoxicities of compounds **1–9**

Compound	IC_{50} (μM)		
	A549	K562	ASPC
1	43.5 ± 6.2	16.6 ± 5.7	>100
2	>100	>100	>100
3	>100	48.5 ± 2.4	>100
4	70.2 ± 2.9	52.3 ± 1.3	>100
5	>100	>100	>100
6	>100	23.5 ± 2.2	>100
7	>100	72.7 ± 3.9	>100
8	>100	>100	92.1 ± 11.4
9	61.1 ± 9.8	28.1 ± 4.7	>100
Taxol	NT	0.9 ± 0.9	>10
5-Flourouracil	19.4 ± 1.2	NT	>50
Cisplatin	28.4 ± 3.3	NT	>50

NT not tested

with **2**. The relative configuration of **3** was elucidated on the basis of the NOE correlations (Fig. 2). The NOE correlations of H-1′ (δ_H 6.60) with H-2′ (δ_H 4.45), and H-2′ with H-3′α (δ_H 2.80) placed them on the same side. The NOE correlations of H-3′β (δ_H 2.67) with H-4′ (δ_H 6.63) indicated that H-3′β and H-4′ were on the opposite side. Compound **3** was determined to be a C-4′ isomer of **2**.

All isolated compounds were evaluated for cytotoxicities against A549, K562, and ASPC cell lines using CCK8 method. The results were summarized in Table 2. Compounds **1**, **3**, **4**, **6**, **7**, and **9** showed inhibition on K562 cell line with IC_{50} value in the range from 16.6 to 72.7 μM. In A549 inhibition assay, **1**, **4**, and **9** showed inhibitory activity with IC_{50} of 43.5, 70.2, and 61.1 μM, respectively. The cytotoxicity of compound **3** ($IC_{50} = 48.53$ μM) against K562 was much stronger than that of **2** ($IC_{50} > 100$ μM). Structures of **3** and **2** differ in the absolute configuration at C-4′. Thus, we infer that *S* absolute configuration at C-4′ in **4** might make great contribution to its inhibitory activity against K562 cell line.

In conclusion, three new phenolic bisabolane sesquiterpenoid esters, aspertenols A–C (**1–3**), together with six known compounds (**4–9**) were isolated and identified from the solid culture of *A. tennesseensis*. The cytotoxicities of **1–14** against A549, K562, and ASPC cell lines were evaluated in vitro. As a result, compounds **1**, **3**, **4**, **6**, **7**, and **9** were found to have inhibitory activity against K562 or A549 cell lines. Compounds **1–3** represent the new type of phenolic bisabolane sesquiterpenoid esters.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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