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•Original article•

Seven drimane-type sesquiterpenoids from an earwig-associated *Aspergillus* sp.

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[ABSTRACT] Drimane-type sesquiterpenoids are widely distributed in fungi. From the ethyl acetate extract of the earwig-derived *Aspergillus* sp. NF2396, seven new drimane-type sesquiterpenoids, named drimanenoids A−G (**1**−**7**), were isolated. Their structures were elucidated by diverse spectroscopic analysis including high-resolution ESI-MS, one- and two-dimensional NMR spectroscopy. Drimanenoids A−F (**1**−**6**) are new members of drimane-type sesquiterpenoid esterified with unsaturated fatty acid side chain at C-6. Drimanenoids C (**3**), D (**4**) and F (**6**) showed antibacterial activity against five types of bacteria with different inhibition diameters. Drimanenoid D (**4**) exhibited moderate cytotoxicity against human myelogenous leukemia cell line K562 with an IC_{50} value of 12.88 ± 0.11 μmol·L^{-1}.

[KEY WORDS] Fungus; Sesquiterpenoids; Drimane-type; Antibacterial; Cytotoxic; *Aspergillus* sp.
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Introduction

Sesquiterpenoids with the drimane skeleton are a class of bioactive metabolites produced by diverse fungi, terrestrial plants and marine animals [1, 2]. Previous research has revealed the presence of a number of rare drimane-type sesquiterpenoid esterified with complex fatty acid side chains, mainly from *Aspergillus* species [3-6]. This type of natural products often displayed a wide range of biological and pharmacological activities, such as antibacterial, antifungal, cytotoxic, antifeedant and plant-growth regulatory effects [1, 3, 5], providing an excellent chance for new drug discovery. As part of an ongoing program for the discovery of structurally novel and bioactive natural products from microorganisms isolated from specialized ecological niches [7-11], we focused on a fungus strain isolated from an earwig collected from Weifang, Shandong Province of China. Chemical investigation of the secondary metabolites produced by this strain resulted in the isolation and identification of six new drimane-type sesquiterpenoid esters, drimanenoids A−F (**1**−**6**), and one new drimane sesquiterpenoid, drimanenoid G (**7**) (Fig. 1). Drimanenoids A−F (**1**−**6**) are new members of drimane-type sesquiterpenoid esters with unsaturated fatty acid moieties at C-6 comprising of one, two or three conjugated olefinic double bonds with a terminal carboxyl or methyl group. The antibacterial activities of these new drimane-type sesquiterpenoids against eight gram-negative bacteria including *Vibrio harveyi*, *Vibrio owensii*, *Vibrio alginolyticus*, *Photobac-*

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These authors have no conflict of interest to declare.

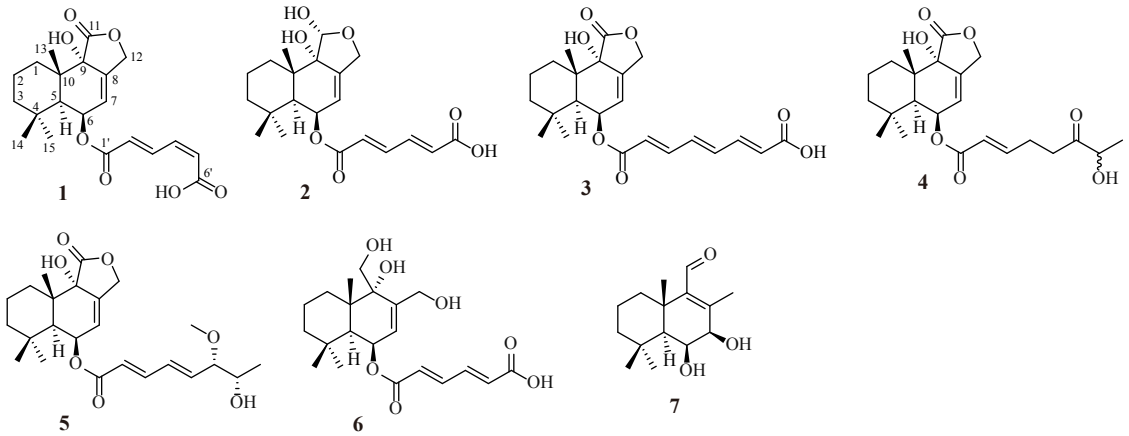

Fig. 1 Structures of drimanenoids A−G (1−7)

terium damselae, *Ralstonia solanacearum*, *Xanthomonas oryzae* pv. *oryzae* (XOO), *Xanthomonas campestris* pv. *mangiferaeindicae* (XCM), and *Escherichia coli* (EC), and two gram-positive bacteria including *Micrococcus luteus* (ML) and methicillin-resistant *Staphylococcus aureus* (MRSA), and the cytotoxic effects of these new natural products against five cell lines including human myelogenous leukemia cell line K562, human gastric carcinoma cell line SGC-7901, human hepatoma carcinoma cell line BEL-7402, human non-small cell lung carcinoma cell line A549, and human cervical carcinoma cell line Hela were evaluated. Herein, we report the isolation, structure identification, and biological evaluation of these new drimane-type sesquiterpenoid natural products.

Results and Discussion

The EtOAc extract of fungal culture was separated by a combination of column chromatography and semi-preparative HPLC purification, obtaining seven new drimane-type sesquiterpenoids (**1**−**7**) (Fig. 1).

Compound **1** was isolated as white solid. Its molecular formula was assigned to be $C_{21}H_{26}O_7$ on the basis of HR-ESI-MS at *m/z* 413.1569 $[M + Na]^+$ (Calcd. 413.1571), ^1H and ^{13}C NMR data (Tables 1 and 2). The ^1H and HSQC NMR spectra of **1** displayed the signals of three tertiary methyl groups [δ_H 1.08 (s, H_3-13), 1.08 (s, H_3-14), 0.93 (s, H_3-15)], five olefinic protons [δ_H 5.83 (br s, H-7), 6.27 (d, J = 15.5 Hz, H-2′), 8.35 (dd, J = 15.5, 11.8 Hz, H-3′), 6.82 (t, J = 11.5 Hz, H-4′), 6.02 (d, J = 11.5 Hz, H-5′)], one oxygenated methylene [δ_H 4.88 and 4.80 (both d, J = 12.7 Hz, H-12)], one oxygenated methine [δ_H 5.60 (br s, H-6)], and one exchangeable proton [δ_H 6.30 (s, OH-9)]. The ^{13}C NMR and DEPT135 spectra exhibited the presence of 21 carbon signals, which was assigned to three carbonyl groups [δ_C 174.3 (C-11), 164.9 (C-1′) and 168.0 (C-6′)], six olefinc carbons, three oxygenated carbons, three methyls and six sp^3 carbons. Comparison of these data with those of the reported compound mono(6-strobilactone-B) ester of (*E*,*E*)-2,4-hexadienedioic acid (**8**) [1] suggested that compound **1** had a similar structure of drimane-type sesquiterpenoid ester with differences on the unsaturated fatty acid side chain. This assumption was evidenced by the HMBC and ^1H-^1H COSY experiments (Fig. 2). The major distinction was that **1** possessed (2*E*,4*Z*)-hexadienedioic acid which was inferred from the NOESY correlation of H-4′ with H-2′ and H-5′ (Fig. 3) and the relatively small coupling constants (J = 11.5 Hz) between H-4′ and H-5′. The relative configuration of the sesquiterpene core in **1** was determined by the NOESY experiment. The NOESY correlations of H-5 with H-6 and OH-9 suggested their locations on the same side, whereas the configuration of C-10 was considered to be on the opposite side by comparison of ^{13}C chemical shifts (δ_C 37.3 for **1** *vs* δ_C 37.3 for **8**) [1]. Thus, the structure of drimanenoid A (**1**) was elucidated as shown in Fig. 1.

Compound **2** was obtained as white solid. The molecular formula of $C_{21}H_{28}O_7$ was deduced from its HR-ESI-MS spectrum at *m/z* 415.1724 $[M + Na]^+$ (Calcd. 415.1727). Detailed comparison of the ^1H and ^{13}C NMR data of **2** with those of compound **8** [1] revealed that the only difference was the presence of a CHOH [δ_H 5.21 (s), 4.83 (brs, OH), δ_C 97.2] instead of a carbonyl group (δ_C 174.7) at C-11 in the five-membered ring in **2**, indicating that the carbonyl group in compound **8** was reduced. This was convinced by the HMBC correlations from H_2-12 to C-11, and from H-11 to C-8, C-9, and C-10 (Fig. 2). The NOESY correlations of H-5 with H-6 and OH-9 and correlations of H_3-13 with OH-11 (Fig. 3) indicated the relative configuration of **2** as shown in Fig. 1.

Compound **3** was isolated as white solid. The HR-ESI-MS (*m/z* 439.1722 $[M + Na]^+$, Calcd. 439.1727) and 1D NMR data indicated the molecular formula to be $C_{23}H_{28}O_7$. Comparison of the NMR data of **3** with those of known compound **8** revealed that they shared the same drimane-type sesquiterpenoid skeleton except for the unsaturated fatty acid side chain, where six conjugated olefinic protons were observed instead of the four present in **8**. The ^1H-^1H COSY correlations of H-3′ with H-2′ and H-4′, and of H-6′ with H-5′ and H-7′ in combination with the HMBC correlations of H-2′ with C-1′, of H-3′ with C-5′, of H-7′ with C-8′, and of H-6

Table 1 ^{1}H NMR data (400 MHz) for compounds 1−7 in DMSO-d_6 (δ in ppm, mult, *J* in Hz)

No.	1	2	3	4	5	6	7
1	1.99 td (13.5, 4.0); 1.85 br d (13.5)	1.87 td (13.5, 3.8); 1.23 br d (13.5)	1.98 td (13.6, 4.0); 1.85 brd (13.6)	1.97 td (13.5, 4.1); 1.84 br d (13.4)	1.97 td (13.3, 4.0); 1.85 br d (13.3)	1.86 m; 1.42 m	2.29 br d (12.8); 0.88 m
2	1.59 m; 1.49 m	1.58 m; 1.43 m	1.60 m; 1.49 m	1.60 m; 1.48 m	1.59 m; 1.47 m	1.59 m; 1.45 m	1.67 m; 1.41 m
3	1.35 br d (12.6); 1.23 m	1.33 m; 1.20 m	1.35 br d (12.1); 1.22 m	1.34 m; 1.21 m	1.34 m; 1.23 m	1.31 m; 1.18 m	1.34 m; 1.13 m
5	2.03 d (4.7)	2.09 d (4.5)	2.03 d (4.8)	2.01 d (4.8)	2.02 d (4.8)	1.99 d (4.2)	1.24 br s
6	5.60 br s	5.60 br s	5.60 br s	5.55 br s	5.59 br s	5.57 t (4.3)	4.04 br s
6-OH							4.57 d (4.1)
7	5.83 br s	5.50 br s	5.82 br s	5.78 br s	5.80 br s	5.78 d (4.3)	3.56 br d (6.5)
7-OH							5.35 d (6.5)
9-OH	6.30 s	6.28 s	6.29 s	6.26 s	6.28 s	4.41 s	
11		5.21 s				3.52 dd (11.3, 3.6); 3.44 dd (11.3, 3.6)	10.05 s
11-OH		4.83 br s				4.63 t (3.6)	
12	4.88 d (12.7); 4.80 d (12.7)	4.39 d (13.0); 4.10 d (13.0)	4.88 d (12.7); 4.79 d (12.7)	4.88 d (12.7); 4.79 d (12.7)	4.88 d (12.8); 4.79 d (12.8)	4.14 d (5.3)	2.09 s
12-OH						4.84 t (5.3)	
13	1.08 s	1.13 s	1.07 s	1.04 s	1.06 s	1.17 s	1.45 s
14	1.08 s	1.08 s	1.08 s	1.06 s	1.08 s	1.05 s	1.16 s
15	0.93 s	0.93 s	0.93 s	0.91 s	0.93 s	0.92 s	0.92 s
2′	6.27 d (15.5)	6.40 overlap	6.14 d (15.3)	5.84 d (15.7)	6.02 d (15.3)	6.39 overlap	
3′	8.35 dd (15.5, 11.8)	7.33 overlap	7.28 dd (15.3, 9.3)	6.89 m	7.24 dd (15.3, 11.0)	7.29 overlap	
4′	6.82 t (11.5)	7.31 overlap	6.89 overlap	2.38 m	6.45 dd (15.5,11.0)	7.31 overlap	
5′	6.02 d (11.5)	6.36 overlap	6.91 overlap	2.74 m	6.16 dd (15.5, 7.0)	6.35 overlap	
6′			7.22 dd (15.3, 9.3)		3.49 m		
6′-COOH		12.64 (br s)				12.63 (br s)	
6′-OCH_3					3.21 s		
7′			6.08 d (15.3)	4.03 m	3.61 m		
7′-OH				5.34 d (5.3)			
8′				1.15 d (6.9)	1.02 d (6.5)		
8′-COOH			12.52 (br s)				

with C-1′ (Fig. 2) confirmed the presence of a side chain of (2′*E*,4′*E*,6*E*′)-7′-carboxyhepta-2′,4′,6′-diene at C-6 *via* an ester bond. The relative configuration of **3** was determined by NOESY correlations of H-5 with H-6 and OH-9 (Fig. 3) and comparison of ^{13}C NMR data with those of **1** and **2**. Therefore, the structure of **3** was assigned as shown in Fig. 1.

Compound **4** was isolated to be white solid and assigned the molecular formula $C_{23}H_{32}O_7$ based on the HR-ESI-MS (*m/z* 443.2037 $[M + Na]^+$, Calcd. 443.2040). The ^{1}H and ^{13}C NMR data for compound **4** indicated a structural similarity to those of **1**−**3** for the change in the side chain. A different side chain was obeserved, as suggested by a spin system from H-2′ to H-5′ deduced from the ^{1}H-^{1}H COSY spectrum. Furthermore, the HMBC correlations of H-3′, H-6 with C-1′, and of H-4′, H_3-8′, OH-7′ with C-6′ in combination with the ^{1}H-^{1}H COSY correlations of H-7′ with H_3-8′ (Fig. 2) supported the (2′*E*)-7′-hydroxy-6-oxyocta-2′-enoyl side chain at C-6. The NOESY (Fig. 3) and ^{13}C NMR data suggested the relative configuration of the sesquiterpene core in **4** to be the same as those of **1** and **3**.

Compound **5** was isolated as white solid. The molecular formula was established to be $C_{24}H_{34}O_7$ based on its HR-ESI-MS (*m/z* 457.2196 $[M + Na]^+$, Calcd. 457.2197) and 1D NMR data. Careful analysis of the NMR data for **5** suggested

Table 2 ^{13}C NMR data (δ in ppm, 100 MHz) for compounds **1**–**7** in DMSO-d_6

No.	1	2	3	4	5	6	7
1	30.7	31.4	30.1	29.6	29.6	31.8	37.9
2	17.4	17.7	17.9	17.4	17.4	18.2	18.7
3	44.5	44.5	44.9	44.4	44.4	44.1	42.5
4	33.3	33.2	33.8	33.3	33.3	33.3	33.4
5	44.2	45.0	44.7	44.1	44.2	44.7	48.1
6	66.4	67.1	66.7	65.8	65.9	67.0	69.5
7	121.1	116.9	121.7	121.3	121.3	119.4	75.5
8	137.0	143.2	137.3	136.7	136.7	145.1	149.8
9	73.1	76.2	73.6	73.1	73.2	74.1	143.0
10	37.3	37.9	37.8	37.3	37.3	40.6	37.4
11	174.3	97.2	174.8	174.4	174.4	61.6	194.3
12	68.2	65.6	68.7	68.2	68.2	60.5	17.2
13	18.2	18.5	18.8	18.3	18.3	18.7	21.1
14	24.5	24.3	24.8	24.3	24.3	24.6	23.6
15	32.2	33.0	32.6	32.1	32.2	32.6	33.3
1′	164.9	164.9	165.5	164.8	165.4	165.1	
2′	127.6	128.1	124.4	121.2	120.9	128.5	
3′	139.9	141.8	144.3	149.7	144.8	141.4	
4′	139.0	140.4	137.3	25.3	130.1	140.6	
5′	127.5	130.2	138.7	34.9	142.2	129.9	
6′	168.0	166.8	142.9	212.7	85.7	166.8	
6′-OCH_3					56.7		
7′			126.4	72.2	68.1		
8′			167.6	19.5	19.3		

that its structure was similar to that of **4**, exhibiting an additional methoxy group and two additional olefinic groups in the side chain. These findings were supported by the ^{1}H-^{1}H COSY correlations from H-4′ to H-8′ and HMBC correlations from H-3′ to C-1′, and from H_3-8′, OCH_3-7′ to C-7′ (Fig. 2), indicating the side chain to be (2′*E*,4′*E*)-7′-hydroxy-6-methoxyocta-2′,4′-dienoyl. This moiety was connected to C-6 from the HMBC correlation of H-6 to C-1′. The relative configurations of C-6′ and C-7′ on the side chain were suggested as *threo*, based on the Δδ value of chemical shifts for C-6′ and C-7′ [12]. The NOESY (Fig. 3) data indicated that the relative configuration of **5** was the same as those of **1**, **3** and **4**.

Compound **6** was isolated as white solid and found to have the molecular formula $C_{21}H_{30}O_7$ based on its HR-ESI-MS spectrum (*m/z* 417.1884 $[M + Na]^+$, Calcd. 417.1884). The 1D NMR data for **6** were comparable to those of **2**, where the differene lay in the replacement of an oxygenated methine group with an oxygenated methylene group [δ_H 3.52 (dd, *J* = 11.3, 3.6, H_a-11), 3.43 (dd, *J* = 11.3, 3.6, H_b-11), δ_C 61.6] and an additional hydroxy group [δ_H 4.83 (t, *J* = 5.3, OH)] in **6**, which was derived from ring-opening of five-membered ring by reduction. This assumption was evidenced by the HMBC correlations from H_2-11 to C-8, C-9 and C-10, and from H_2-12 with C-7, C-8 and C-9 (Fig. 2). Also the HMBC cross-peak between OH-12 and C-8 was observed. The relative configuration of **6** was determined on the basis of biosynthetic consideration and NOESY data.

Compound **7** was isolated as white solid and had the molecular formula $C_{15}H_{23}O_3$ on the basis of a HR-ESI-MS peak at *m/z* 251.1656 $[M - H]^-$. Detailed analysis of the ^{1}H, ^{13}C, DEPT135 and HSQC NMR data of **7** revealed that the structure of **7** was closely related to the reported drim-8-en-6b,7a,11-triol [5], possessing a drimane-type sesquiterpenoid skeleton. The major difference was that **7** contains an additional aldehyde group [δ_H 10.05 (1H, s), δ_C 194.3] instead of a CH_2OH group, which is located at C-11 on the basis of the HMBC correlations from H-11 to C-8 and C-10 (Fig. 2). Further comprehensive analysis of the HMBC and ^{1}H-^{1}H COSY spectra of **7** allowed the determination of the planar structure.

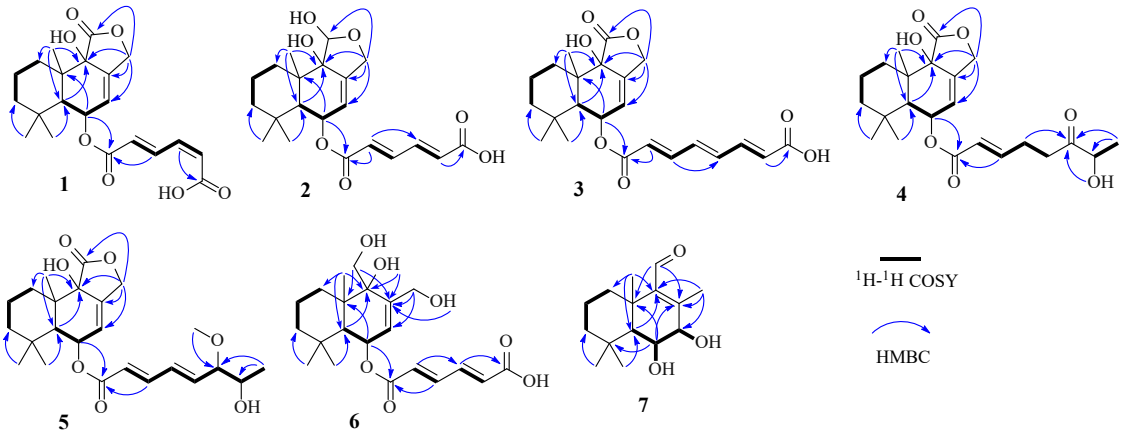

Fig. 2 Key HMBC and ^{1}H-^{1}H COSY correlations of drimanenoids A−G (1−7)

The relative configuration of **7** was deduced from the NOESY correlations of H_3-15 with H-5 and H-6, of H-6 with H-5 and H-7, of H-5 with H-7, and of H_3-13 with H_3-14 (Fig. 3). Thus, the structure of **7** was elucidated as shown in Fig. 1.

Drimane-type sesquiterpenoid esters have been reported to exert antibacterial and cytotoxic activities [1]. The new drimane-type sesquiterpenoid esters **1**−**4** and **6** were assessed for their antibacterial activities against eight gram-negative bacteria, namely *V. harveyi*, *V. owensii*, *V. alginolyticus*, *P. damselae*, *R. solanacearum*, XOO, XCM, and EC, and two gram-positive bacteria, including ML and MRSA using the agar diffusion method. The results were shown in Table 3. Among these tested compounds, compound **1** exhibited inhibitory activity against ML, EC, XOO and XCM. Compound **2** only showed inhibitory activity against EC and XCM. Meanwhile, compounds **3**, **4** and **6** possessed the broadest spectra of antibacterial activity against the gram-positive bacteria including ML and MRSA, and the gram-negative bacteria including EC, XOO and XCM. However, all of these tested compounds were inactive against the gram-negative bacteria, namely *V. harveyi*, *V. owensii*, *V. alginolyticus*, *P. damselae* and *R. solanacearum*. Furthermore, compounds **1**−**4** and **6** were also evaluated for their abilities to inhibit the growth of five carcinoma cells, including K562, SGC-7901, BEL-7402, A549 and Hela cell lines using MTT assay. Among them, only compound **4** displayed moderate cytotoxicity against K562 cells with an IC_{50} value of 12.88 ± 0.11 μmol·L^{-1} (3.08 ± 0.05 μmol·L^{-1} for the positive drug), while the IC_{50} values of other compounds were more than 40 μmol·L^{-1}.

Experimental

General experimental procedures

The HR-ESI-MS data of the isolated compounds were obtained on an Agilent 6210 TOF LC-MS instrument (Agilent Technologies Inc., Santa Clara, CA, USA). The optical rotation values were recorded in methanol on a Rudolph Autopol IV automatic polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). The UV and IR spectra were measured on a Hitachi U-3000 spectrophotometer and a Nexus 870 FT-IR spectrometer, respectively. The 1D and 2D NMR spectra (including ^{1}H, ^{13}C, DEPT135, HSQC, HMBC, ^{1}H-^{1}H COSY and NOESY) were obtained on a Bruker Avance III 400 MHz NMR spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR). The chemical shifts of ^{1}H and ^{13}C NMR data were given in ppm and referenced to the solvent signal (DMSO-d_6, δ_H 2.50 and δ_C 39.52). Coupling constants (*J*) were reported in Hz. Semi-preparative HPLC was performed on an Agilent Technologies 1260 Infinity II instrument equipped with an Agilent Eclipse C_{18} column (5 μm, 250 mm × 9.4 mm). MPLC fractionation was performed on a Biotage Isolera One using a Biotage SNAP Catridge C_{18} column (120 g). Sephadex LH-20 gel (Pharmacia Biotech, Sweden) was used for column chromatography (CC). Precoated glass plates (silica gel GF_{254}, 10–20 μm, Qingdao Marine Chemical Factory, Qingdao, China) were employed for thin layer chromatography (TLC).

Fungus material, cultivation, fermentation and isolation

The fungus strain *Aspergillus* sp. NF2396 was isolated from an earwig collected from the suburb of Weifang, Shan-

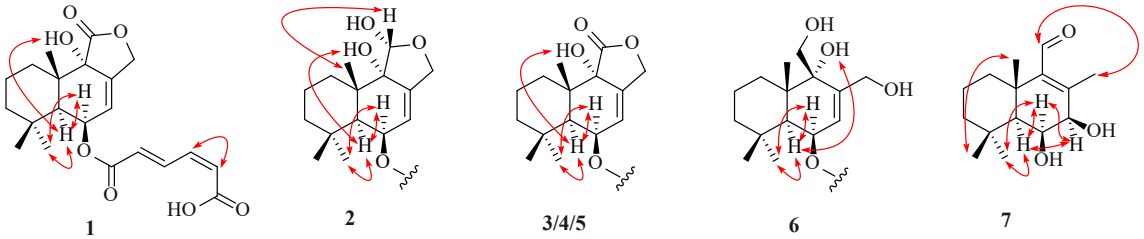

Fig. 3 Key NOESY correlations of drimanenoids A−G (1−7)

Table 3 Antibacterial activities of compounds 1–4 and 6 (inhibition diameters: mm)

Compounds	ML	MRSA	EC	XOO	XCM
1	4.50 ± 0.40	–	1.00 ± 0.81	2.00 ± 0.00	3.66 ± 0.23
2	–	–	4.83 ± 0.23	–	3.83 ± 0.23
3	4.66 ± 0.23	2.66 ± 0.47	4.83 ± 0.47	5.00 ± 0.23	4.00 ± 1.08
4	2.66 ± 0.47	4.66 ± 0.47	2.00 ± 0.81	1.00 ± 0.00	1.33 ± 0.94
6	4.66 ± 0.84	5.00 ± 0.00	3.66 ± 0.94	4.75 ± 0.70	4.50 ± 0.70
Kanamycin*	14.33 ± 1.24	14.66 ± 0.62	9.66 ± 1.24	14.50 ± 0.81	12.66 ± 1.24

ML: *Micrococcus luteus*; MRSA: methicillin-resistant *Staphylococcus aureus*; EC: *E. coli*; XOO: *Xanthomonas oryzae* pv. *oryzae*; XCM: *Xanthomanas campestris* pv. *mangiferaeindicae*; "–" no activity; "*" positive control: 500 μg per disc; compounds **1**–**6**: 50 μg per disc

dong Province, China and identified by one of the authors (SALMAN Khan from Nanjing University). The strain was cultivated on potato dextrose agar (PDA) medium at 30 °C for 5 days. Then small agar plugs with mycelia were directly inoculated into 1 L Erlenmeyer flasks containing 400 mL ME liquid medium (consisting of 20 g·L^{-1} malt extract, 20 g·L^{-1} sucrose, and 1 g·L^{-1} peptone), which were cultivated at 30 °C for 14 days on a rotary shaker at 140 r·min^{-1}. After fermentation, the broth was filtered to yield the filtrate and biomass. Then the filtrate was extracted with an equal volume of ethyl acetate (EtOAc) three times. The final extraction solvent was combined and evaporated to dryness under reduced pressure to yield a crude extract. Then the crude extract was seperated by a medium-pressure liquid chromatography (MPLC) system equipped a Biotage SNAP Ultra C_{18} 120 g column using a linear gradient system from 10% to 100% (*V*/*V*) methanol/water to give seven fractions (A–F). Then, the fractions were fractionated by Sephadex LH-20 chromatography (MeOH) and further purified by semi-preparative HPLC. Compounds **1** (2.2 mg, t_R 21.0 min), **2** (5.4 mg, t_R 13.5 min), **3** (6.5 mg, t_R 22.0 min), **4** (3.1 mg, t_R 23.5 min) and **6** (2.1 mg, t_R 9.5 min) were purified from fraction E by semi-preparative HPLC using 50% MeCN in H_2O at a flow rate of 2.2 mL·min^{-1}. Compound **5** (2.4 mg, t_R 10.5 min) was purified from fraction F by semi-preparative HPLC using 62% MeCN in H_2O at a flow rate of 2.2 mL·min^{-1}. Compound **7** (8.5 mg, t_R 14.5 min) was purified from fraction D by semi-preparative HPLC using 44% MeCN in H_2O at a flow rate of 2.2 mL·min^{-1}.

Drimanenoid A (**1**): white solid; $[\alpha]_D^{25}$ −198.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 267.0 (3.02) nm; CD (1.28 × 10^{-3} mol·L^{-1} in MeOH) λ_{max} ($\Delta\varepsilon$): 194 (+3.82), 210 (−22.74) nm; IR (KBr) ν_{max} 3451, 2924, 1774, 1702, 1677, 1655, 1561, 1459, 1168, 1008, 661 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HR-ESI-MS *m/z* 413.1569 $[M + Na]^+$ (Calcd. for $C_{21}H_{26}O_7Na$, 413.1571).

Drimanenoid B (**2**): white solid; $[\alpha]_D^{25}$ −64.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 266.0 (2.82) nm; CD (1.28 × 10^{-3} mol·L^{-1} in MeOH) λ_{max} ($\Delta\varepsilon$): 191 (+8.62), 215 (−6.91) nm; IR (KBr) ν_{max} 3448, 2931, 1751, 1686, 1655, 1459, 1148, 1025, 645 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HR-ESI-MS *m/z* 415.1724 $[M + Na]^+$ (Calcd. for $C_{21}H_{28}O_7Na$, 415.1727).

Drimanenoid C (**3**): white solid; $[\alpha]_D^{25}$ −228.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 284.0 (3.21), 299.0 (3.19) nm; CD (1.20 × 10^{-3} mol·L^{-1} in MeOH) λ_{max} ($\Delta\varepsilon$): 192 (−2.68), 195 (−3.04), 210 (−24.74) nm; IR (KBr) ν_{max} 3446, 2930, 1794, 1774, 1735, 1655, 1638, 1459, 1008 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HR-ESI-MS *m/z* 439.1722 $[M + Na]^+$ (Calcd. for $C_{23}H_{28}O_7Na$, 439.1727).

Drimanenoid D (**4**): white solid; $[\alpha]_D^{25}$ −145.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 263.0 (2.57) nm; CD (1.19 × 10^{-3} mol·L^{-1} in MeOH) λ_{max} ($\Delta\varepsilon$): 193 (−11.87), 196 (−1.04), 216 (−33.38) nm; IR (KBr) ν_{max} 3423, 2922, 1718, 1701, 1685, 1560, 1458, 1074, 650 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HR-ESI-MS *m/z* 443.2037 $[M + Na]^+$ (Calcd. for $C_{23}H_{32}O_7Na$, 443.2040).

Drimanenoid E (**5**): white solid; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HR-ESI-MS *m/z* 457.2196 $[M + Na]^+$ (Calcd. for $C_{24}H_{34}O_7Na$, 457.2197).

Drimanenoid F (**6**): white solid; $[\alpha]_D^{25}$ −104.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 267.0 (3.03) nm; CD (1.27 × 10^{-3} mol·L^{-1} in MeOH) λ_{max} ($\Delta\varepsilon$): 195 (+2.95), 207 (−10.87) nm; IR (KBr) ν_{max} 3422, 2956, 1774, 1702, 1655, 1647, 1578, 1499, 1080, 650 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HR-ESI-MS *m/z* 417.1884 $[M + Na]^+$ (Calcd. for $C_{21}H_{30}O_7Na$, 417.1884).

Drimanenoid G (**7**): white solid; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HR-ESI-MS *m/z* 251.1656 $[M - H]^-$ (Calcd. for $C_{15}H_{23}O_3$, 251.1653).

Antibacterial activity test

Compounds **1**–**4** and **6** were evaluated for their inhibitory activity against the gram-negative bacteria including *V. harveyi*, *V. owensii*, *V. alginolyticus*, *P. damselae*, *R. solanacearum*, XOO, XCM, and EC, and the gram-positive bacteria including ML and MRSA according to the previously reported methods [13]. The antibacterial activity was performed *in vitro* using the agar diffusion method with 7 mm paper discs, where 500 μg of kanamycin was used as a positive control. Due to the low amount of the tested compounds, each 7 mm paper disc only contained 50 μg of the corresponding compound in the test.

Cytotoxic activity test

The cytotoxic activity of compounds **1**–**4** and **6** were

screened *in vitro* towards human myelogenous leukemia cell line K562, human gastric carcinoma cell line SGC-7901, human hepatoma carcinoma cell line BEL-7402, human non-small cell lung carcinoma cell line A549, and human cervical carcinoma cell line Hela using the previously reported methods [14]. The *cis*-platinum was used as the positive drug.

Supplementary data

Supplementary material related to this article can be requested by sending E-mails to the corresponding authors.

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