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Wentinoids A–F, six new isopimarane diterpenoids from *Aspergillus wentii* SD-310, a deep-sea sediment derived fungus†

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Six new isopimarane-type diterpenoid derivatives, wentinoids A–F (1–6), along with a known congener (7), were identified from a culture of *Aspergillus wentii* SD-310, a fungus isolated from a deep-sea sediment sample. The structures of these compounds were determined by analysis of spectroscopic data, and their absolute configurations were established by single crystal X-ray analysis or TDDFT-ECD calculations. This is the first time to report the isolation of isopimarane analogues from the fungal species *Aspergillus wentii*. Among these compounds, wentinoid A (1) possesses a unique 20-acetal and a 7,20-oxa-bridged functionality, while wentinoid B (2) contains an unusual 8,20-lactone-bridged scaffold. Compound 1 exhibited potent inhibitory activities against four plant-pathogenic fungi.

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Introduction

Diterpenoids of the isopimarane-type comprise a structurally diverse and functionally noteworthy group of natural products, which are much more widely distributed in plants^{1–3} than in fungi.⁴ A number of isopimarane analogues have been proven to act as ecologically and biologically functional substances. Typical examples of isopimarane diterpenoids with ecological-related activities are pedinophyllols, which were isolated from the Chinese liverwort *Pedinophyllum interruptum* and exhibited germination inhibition for *Arabidopsis thaliana* seeds.⁵ Some isopimarane derivatives displayed cytotoxicity,^{6,7} anti-microbial,^{8,9} anti-malarial,¹⁰ anti-inflammatory,¹¹ and anti-virus¹² properties as well as inhibitory activities against acetylcholinesterase,¹³ α -glucosidase,³ and nitric oxide production.² As part of our ongoing efforts to discover bioactive metabolites from marine-derived fungi,^{14–16} we recently focused our attention on *Aspergillus wentii* SD-310, a fungal strain obtained from a deep-sea sediment sample. As a result, a series of 20-nor-isopimarane derivatives were isolated and identified.^{15,16} Further work on this fungus has now resulted in the isolation of six new

isopimarane-type diterpenoid derivatives, wentinoids A–F (1–6), together with a known compound 7. The planar structures of compounds 1–6 were established on the basis of spectroscopic analysis, and the absolute configurations were confirmed by single-crystal X-ray diffraction analysis or ECD calculations. These compounds represents the first example of isopimarane analogues isolated from the fungal species *A. wentii*, with wentinoid A (1) belongs to a rarely described class of tetracyclic isopimaranes, which contain an oxa-bridge-ring. In contrast to other related congeners, compound 1 possesses a unique 20-acetal moiety and 7,20-oxa-bridged functionality. Meanwhile, wentinoid B (2) represents a tetracyclic isopimarane and contains an unusual 8,20-lactone-bridged scaffold. The anti-microbial activities of the isolated compounds against 11 human-, and aqua-pathogenic bacteria and seven plant-pathogenic fungi were evaluated. Details of the isolation, structure elucidation, and biological activities of compounds 1–7 are described herein.

Results and discussion

The mycelia and culture broth of *A. wentii* SD-310 were extracted with MeOH and EtOAc, respectively, to obtain two extracts, which were combined and purified by a combination of column chromatography (CC) on Si gel, Lobar LiChroprep RP-18, and Sephadex LH-20, to yield compounds 1–7 (Fig. 1).

Wentinoid A (1) was initially obtained as colorless oily powder. Its molecular formula was determined to be C₂₁H₃₂O₄ by HRESIMS, indicating six degrees of unsaturation. The ¹H and ¹³C NMR, DEPT, and HMQC spectroscopic data (Tables 1 and 2) revealed that 1 contained a tetra-substituted olefin (δ_C 131.4, C-8; δ_C 141.6, C-9), a vinyl group (δ_C 145.8, δ_H 6.03, dd, J = 17.7,

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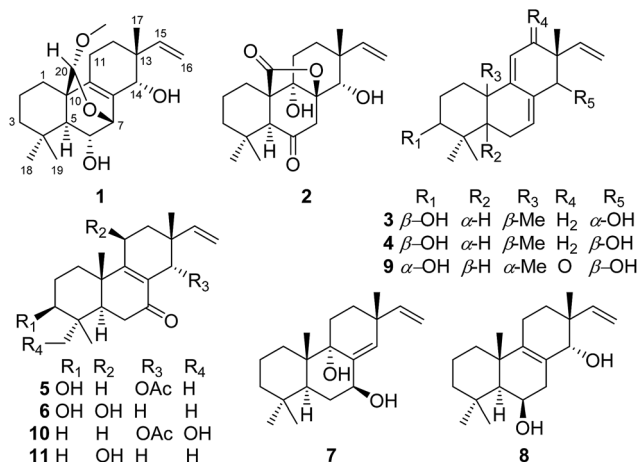


Fig. 1 Chemical structures of compounds 1–7 and reference compounds 8–11.

10.2 Hz, CH-15; δ_{C} 111.4, δ_{H} 4.98, d, $J = 17.7$ Hz, 4.96, d, $J = 10.2$ Hz, CH₂-16), an acetal carbon (δ_{C} 97.7, C-20), three oxygenated methines ($\delta_{\text{C/H}}$ 68.0/3.68, C-6; $\delta_{\text{C/H}}$ 72.7/4.14, C-7; $\delta_{\text{C/H}}$ 71.1/3.49, C-14), and a methoxy group ($\delta_{\text{C/H}}$ 54.2/3.13, C-21). The protons of methoxy group (H₃-21) showed HMBC correlation to C-20, which implied a connection between the methoxy and

C-20 (Fig. 2). The attachment of the *gem*-dimethyl groups (C-18 and C-19) to the quaternary carbon C-4 were identified by their mutual HMBC correlations, along with correlations from H₃-18 and H₃-19 to C-3, C-4, and C-5. Furthermore, signals for three quaternary carbons, an sp³ methine, five aliphatic methylenes, and a singlet methyl were also observed from the NMR spectra.

Comparison of the NMR data of 1 with that of kaempulchraol C (8),¹ an isopimarane-type diterpenoid isolated from rhizomes of *Kaempferia pulchra*, revealed that the structures of these two compounds are very similar. However, signals for the bridgehead methyl (C-20) and the methylene group (C-7) in 8 were absent in the NMR spectra of 1. Instead, resonances for an acetal carbon (C-20) and an oxygenated methine group (C-7) were observed in the NMR spectra of 1. In addition, C-7 and C-20 connected each other *via* an oxygen atom, and this deduction was verified by the key HMBC correlations from H-7 to C-5, C-9, and C-20 and from H-20 to C-1, C-5, C-7, and C-9. The planar structure of 1 was fully defined by the HMBC data as shown in Fig. 2.

The relative configuration of 1 was assigned by analysis of the NOESY data (Fig. 3). While NOE correlations from H-5 to H-3 α , 6-OH, H-7, H-11 α , and H₃-18 and from H-15 to H-11 α and H-12 α indicated the cofacial orientation of these groups, correlations from H-6 to H₃-19 and H-20, from H-11 β to H₃-17, and

Table 1 ¹H NMR data for compounds 1–6 (500 MHz, J in Hz, measured in DMSO-*d*₆)

Position	1	2	3	4	5	6
1	α 1.49 m β 1.86 m	α 1.35 td (13.6, 3.6) β 1.78 d (13.6)	α 1.34 td (12.9, 5.4) β 1.78 dt (12.9, 5.4)	α 1.34 td (12.8, 5.4) β 1.79 dt (12.8, 3.3)	α 1.44 m β 1.78 m	α 1.35 td (13.1, 3.6) β 2.45 m
2	1.45 m	α 1.46 m β 1.89 dtt (13.6, 13.6, 3.6)	1.54 m	1.55 m	1.60 m	α 1.55 m β 1.61 m
3	α 1.17 m β 1.41 m	α 1.08 td (13.6, 3.6) β 1.30 d (13.6)	2.99 m	2.99 m	3.11 m	3.05 m
5	0.75 d (3.5)	2.84 s	1.08 dd (11.6, 4.5)	1.05 dd (11.7, 4.5)	1.62 dd (14.3, 4.2)	1.48 m
6	3.68 m	—	α 2.13 m β 2.05 m	α 2.05 m β 2.00 m	α 2.34 dd (18.2, 4.2) β 2.43 dd (18.2, 14.3)	α 2.27 dd (18.6, 3.3) β 2.48 dd (18.6, 3.5)
7	4.14 d (4.2)	α 3.35 d (16.4) β 2.28 d (16.4)	5.77 brs	5.70 brs	—	—
11	α 2.17 d (17.7) β 1.75 ddd (17.7, 11.6, 5.4)	α 1.69 dt (14.8, 3.6) β 1.50 dd (14.8, 3.6)	5.26 brs	5.28 brs	α 2.49 m β 2.27 m	4.38 brs
12	α 1.67 td (11.6, 5.4) β 1.29 m	α 2.08 td (13.6, 3.6) β 1.18 d (13.6)	α 2.20 dd (17.8, 4.3) β 1.97 dd (17.8, 4.3)	α 1.93 dd (18.0, 4.6) β 2.12 m	α 1.75 m β 1.48 m	α 1.80 dd (12.0, 6.0) β 1.46 dd (12.0, 6.0)
14	3.49 s	3.22 s	3.71 s	3.54 s	5.58 s	α 2.10 d (17.2) β 1.97 d (17.2)
15	6.03 dd (17.7, 10.2)	5.98 dd (17.5, 11.1)	5.85 dd (16.9, 10.1)	5.73 dd (17.6, 10.9)	5.79 dd (17.2, 11.2)	5.69 dd (17.5, 10.8)
16	4.98 d (17.7) 4.96 d (10.2)	4.93 d (17.5) 4.92 d (11.1)	4.93 dd (16.9, 1.6) 4.90 dd (10.1, 1.6)	4.95 d (17.6) 4.87 d (10.9)	4.97 dd (17.2) 4.96 d (11.2)	4.88 d (10.8) 4.80 d (17.5)
17	0.87 s	0.94 s	0.95 s	0.91 s	0.78 s	0.99 s
18	0.94 s	1.12 s	0.88 s	0.88 s	0.89 s	0.87 s
19	0.92 s	0.95 s	0.78 s	0.78 s	0.79 s	0.78 s
20	4.80 s	—	0.88 s	0.88 s	1.07 s	1.25 s
21	3.13 s	—	—	—	—	—
22	—	—	—	—	1.83 s	—
3-OH	—	—	4.41 s	4.42 s	4.52 d (4.3)	4.47 brs
6-OH	4.61 brs	—	—	—	—	—
11-OH	—	—	—	—	—	4.92 d (6.0)
14-OH	3.95 brs	—	4.61 s	4.56 s	—	—



Table 2 ^{13}C NMR data for compounds 1–6 (125 MHz, measured in $\text{DMSO}-d_6$)

Position	1	2	3	4	5	6
1	25.9 CH_2	24.1 CH_2	34.4 CH_2	35.0 CH_2	33.6 CH_2	34.6 CH_2
2	17.7 CH_2	16.9 CH_2	26.8 CH_2	27.4 CH_2	27.0 CH_2	27.0 CH_2
3	40.7 CH_2	41.9 CH_2	76.3 CH	76.8 CH	75.6 CH	76.1 CH
4	32.9 qC	32.4 qC	38.0 qC	38.5 qC	38.4 qC	38.5 qC
5	54.7 CH	56.3 CH	47.5 CH	48.0 CH	47.5 CH	49.8 CH
6	68.0 CH	207.4 qC	22.5 CH_2	23.0 CH_2	34.7 CH_2	32.7 CH_2
7	72.7 CH	48.1 CH_2	121.0 CH	123.8 CH	196.7 qC	199.9 qC
8	131.4 qC	85.4 qC	134.0 qC	134.7 qC	127.4 qC	128.9 qC
9	141.6 qC	74.5 qC	144.6 qC	144.4 qC	170.0 qC	163.3 qC
10	44.0 qC	53.8 qC	35.5 qC	36.0 qC	39.0 qC	39.4 qC
11	21.3 CH_2	26.0 CH_2	113.5 CH	114.6 CH	21.4 CH_2	65.3 CH
12	28.1 CH_2	24.3 CH_2	35.7 CH_2	34.2 CH_2	25.4 CH_2	44.0 CH_2
13	39.1 qC	39.8 qC	40.1 qC	40.3 qC	38.1 qC	34.8 qC
14	71.1 CH	72.5 CH	74.7 CH	75.1 CH	67.8 CH	34.0 CH_2
15	145.8 CH	147.7 CH	142.4 CH	145.2 CH	144.0 CH	145.5 CH
16	111.4 CH_2	110.4 CH_2	111.7 CH_2	111.6 CH_2	112.4 CH_2	111.2 CH_2
17	20.4 CH_3	21.5 CH_3	23.2 CH_3	21.0 CH_3	20.9 CH_3	27.6 CH_3
18	33.3 CH_3	32.4 CH_3	27.4 CH_3	27.9 CH_3	27.2 CH_3	27.6 CH_3
19	22.4 CH_3	19.1 CH_3	15.2 CH_3	15.7 CH_3	15.3 CH_3	15.5 CH_3
20	97.7 CH	176.7 qC	20.6 CH_3	21.0 CH_3	18.5 CH_3	18.5 CH_3
21	54.2 CH_3	—	—	—	168.8 qC	—
22	—	—	—	—	20.9 CH_3	—

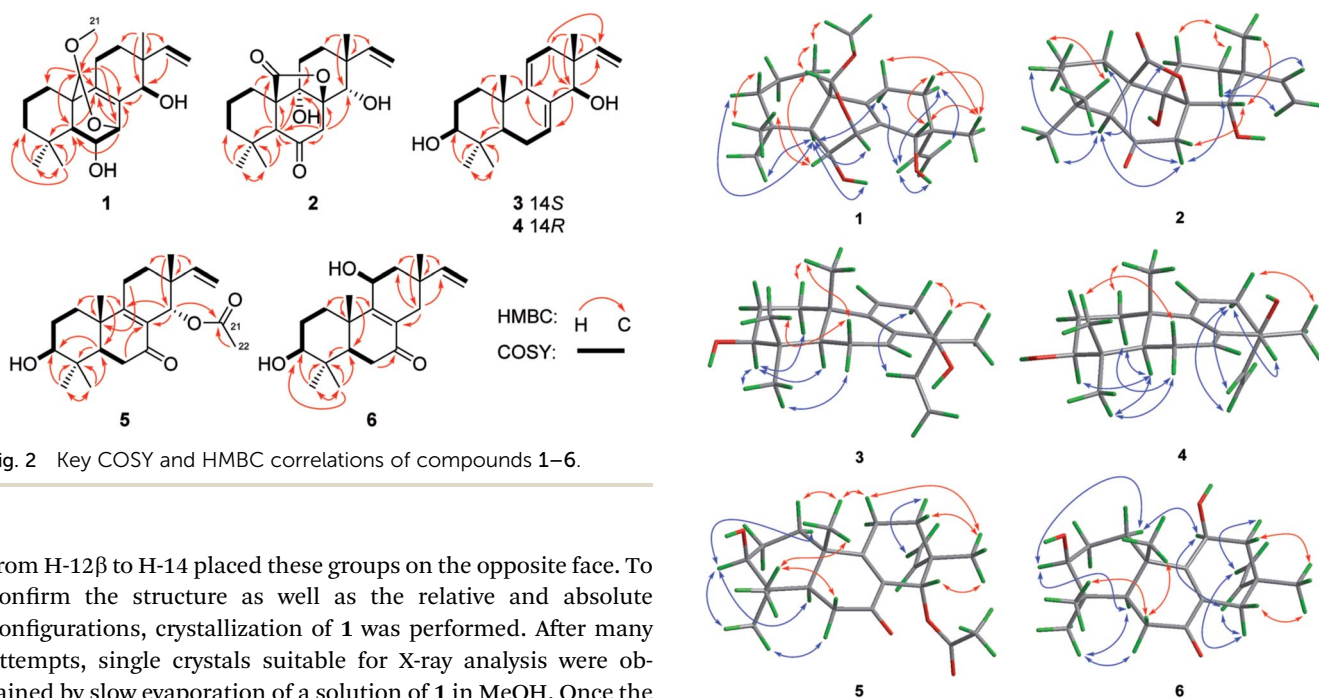


Fig. 2 Key COSY and HMBC correlations of compounds 1–6.

from H-12 β to H-14 placed these groups on the opposite face. To confirm the structure as well as the relative and absolute configurations, crystallization of **1** was performed. After many attempts, single crystals suitable for X-ray analysis were obtained by slow evaporation of a solution of **1** in MeOH. Once the X-ray crystallographic experiment was conducted, the structure and absolute configuration of **1** were confidently assigned as depicted (Fig. 4).

Wentinoid B (**2**) was obtained as colorless oil. The molecular formula $\text{C}_{20}\text{H}_{28}\text{O}_5$, implying seven degrees of unsaturation, was established by HRESIMS. The ^1H NMR spectrum displayed typical signals for a vinyl group (H-15 and H₂-16), three singlet methyls (H₃-17–H₃-19), a pair of methylene protons (H₂-7), an oxygenated methine (H-14), an aliphatic methine (H-5), and other ten aliphatic protons (Table 1). Its ^{13}C NMR data (Table 2) exhibited the presence of 20 carbon

Fig. 3 Key NOESY correlations of 1–6.

signals, which were sorted by DEPT and HSQC spectrum into three methyls, seven methylenes (one olefinic), three methines (one oxygenated and one olefinic), and seven quaternary (one lactone, one ketone and one oxygenated aliphatic quaternary) carbons. Interpretation of the COSY and HSQC spectra of **2** resulted in the elucidation of two discrete proton spin-coupling systems corresponding to a $-\text{CH}_2-\text{CH}_2-\text{CH}_2-$ unit (C-1 through C-3) and a $-\text{CH}_2-\text{CH}_2-$ unit (C-11 and C-12). Key



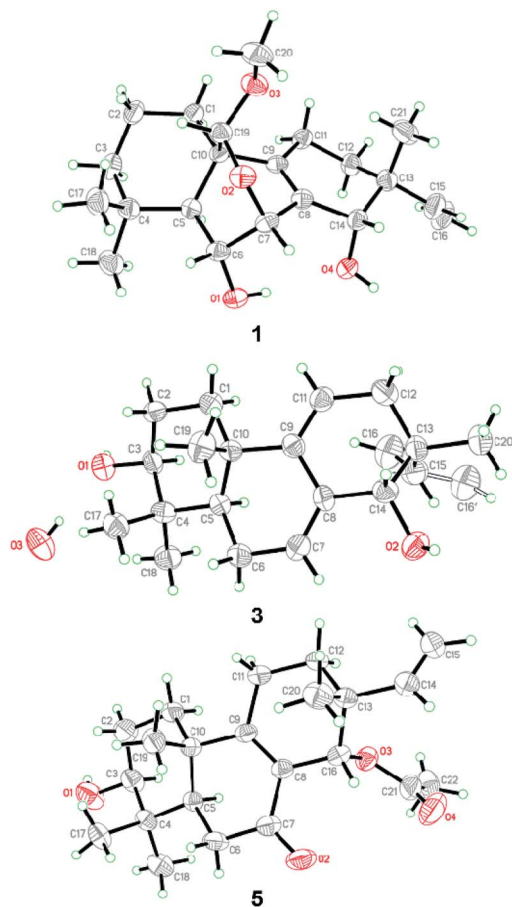


Fig. 4 X-ray crystallographic structures of compounds **1**, **3**, and **5** (note: different numbering systems are used for the structures in the text).

HMBC correlations shown in Fig. 2 suggested the presence of an isopimarane-type diterpenoid skeleton for **2**. The ketone and two hydroxy groups were placed at C-6, C-9 and C-14, respectively, as supported by HMBC correlations from H-5 and H₂-7 to C-6 and C-9 and from H₂-7 and H₃-17 to C-14. The remaining oxygen atom and one degree of unsaturation, together with the consideration of the strongly deshielded nature of C-8 (δ_{C} 85.4) suggesting the presence of a lactone bridge between C-8 and C-10 *via* C-20. Thus, the entire structure of compound **2** was identified as shown in Fig. 1.

The relative configuration of **2** was determined by NOESY experiment and by comparison of 1D NMR data of **2** with related analogues. Key NOE correlations from H-5 to H-7 α and H₃-18, from H-7 α to H-11 α and H-12 α and from H-12 α to H-15 placed these protons on the same face of the molecule. Moreover, NOE correlations from H-7 β to H-14 and from H₃-17 to H-11 β suggested these groups on the opposite face of the molecule. The assignment of the β -orientation of the lactone group was established by analysis of the magnetic anisotropic effect as the method reported by the previous ref. 7. The deshielding effect from the carbonyl at C-20 resulted in downfielded chemical shift of H-2 β . The chemical shift of C-9 (δ_{C} 74.5) in ¹³C-NMR was consistent with those of related

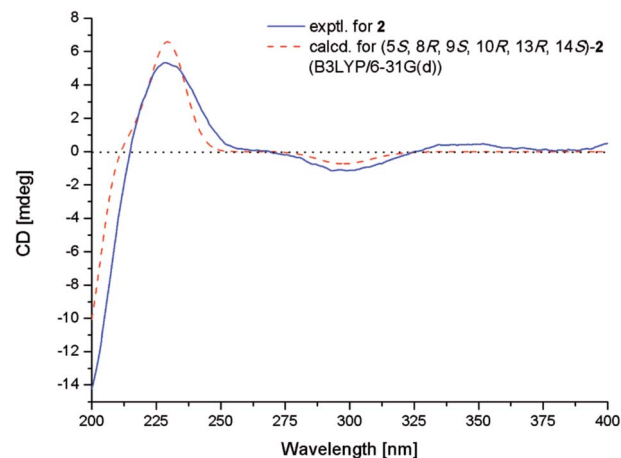


Fig. 5 Experimental and calculated ECD spectra of **2**.

diterpenoids with an α -OH at C-9 (δ_{C} 73.6–75.6).⁷ In order to determine the absolute configuration of **2**, conformational analysis and TDDFT-ECD calculations were performed on the arbitrarily chosen of (5*S*,8*R*,9*S*,10*R*,13*R*,14*S*)-**2**. The DFT reoptimization of the initial MM + conformers of the selected enantiomer at the B3LYP/6-31G(d) level in gas phase afforded minimum energy conformers. The TDDFT-ECD spectra of the conformers were calculated with B3LYP and the 6-31G(d) basis set. The computed ECD spectra of (5*S*,8*R*,9*S*,10*R*,13*R*,14*S*)-**2** matched well to the experimental ECD spectrum, which showed positive cotton effects (CEs) near 228 and 344 nm and negative CE near 299 nm (Fig. 5).

Wentinoid C (**3**) was originally obtained as colorless oily powder. The HREIMS data of **3** determined the molecular formula C₂₀H₃₀O₂. The ¹H and ¹³C NMR data (Tables 1 and 2) are very similar to those of *ent*-3 β ,14 α -hydroxypimara-7,9(11),15-triene-12-one (**9**),¹⁷ implying the same carbon skeleton for both compounds. However, resonance at δ_{C} 202.2 for the ketone group at C-12 in **9** was not observed in that of **3**. Instead, signals for a methylene group at δ_{C} 35.7 were found in the ¹³C-NMR spectra of **3**. Compared to **9**, obvious upfielded shift for C-11, C-13, and C-15, and downfielded shift for C-17 in the ¹³C NMR spectrum of **3** were also observed. These data indicated the replacement of a ketone group in **9** by a methylene group in **3**, which was consistent with the molecular formula. The COSY correlations from H₂-11 to H₂-12 as well as key HMBC correlations from H₂-12 to C-9, C-14, and C-15 and from H₃-17 to C-12 verified the above deduction (Fig. 2).

NOE correlations from H-1 β to H₃-20, from H-6 β to H₃-19 and H₃-20, and from H-14 to H-12 β , H₃-17 and H₃-20 revealed that these protons are on the same side, while correlations from H-3 to H-1 α , H-5, and H₃-18 and from H-6 α to H₃-18 indicated that they are on the other side. To unequivocally determine the absolute configuration, single crystals were cultivated upon slow evaporation of the solvent (MeOH) and a Cu/K α X-ray diffraction analysis was conducted. The final refinement of the X-ray data resulted in a 0.0(14) Flack parameter, allowing for the unambiguous assignment of the absolute configuration as shown in Fig. 4.



Wentinoid D (**4**) was obtained as colorless oil. The molecular formula was determined as $C_{20}H_{30}O_2$, same as that of **3**, on the basis of HREIMS data. The similar UV absorptions to those of compound **3** implied that **4** was an analogue of **3**. The NMR spectra of **4** were also close to that of **3**. Inspection of the 1H and ^{13}C NMR data (Tables 1 and 2) suggested that **4** is a diastereomer of **3**, epimeric at C-14. This was supported by the minor differences on the chemical shifts for C-7, C-12, C-15, and C-17 in **4**, which were from γ -gauche effect caused by the inversion of the absolute configuration at C-14. NOE correlations from H-12 β to H₃-17 and from H-12 α to H-14, H-15, and H₂-16 confirmed this deduction (Fig. 3). The structure of **4** was thus assigned as shown in Fig. 1.

Wentinoid E (**5**), initially obtained as colorless oily powder, had the molecular formula $C_{22}H_{32}O_4$ (seven degrees of unsaturation) as determined by HRESIMS data. Detailed comparison of the NMR data of **5** with those of sorgerolone (**10**)¹³ revealed that compound **5** had the same basic structure as **10**. However, signals for the methylene group resonating at δ_C 34.4 and δ_H 1.35/1.56 (CH₂-3) and those of the hydroxymethyl group at δ_C 70.6 and δ_H 3.15/3.43 (CH₂-18) in **10** disappeared in the NMR spectra of **5**. Instead, signals for an oxygenated methine group at δ_C 75.6 and δ_H 3.11 (CH-3) and a methyl at δ_C 27.2 and δ_H 0.89 (CH₃-18) were observed (Tables 1 and 2). The above observation suggested that compound **5** was 3-hydroxy-18-dehydroxy derivative of **10**. The cross-peaks from H₂-2 to H₂-1 and H-3 and from H-3 to 3-OH in the COSY spectrum as well as the correlations from H-5 to C-3, from H₃-18 and H₃-19 to C-3, C-4, and C-5 in the HMBC spectrum confirmed the above deduction (Fig. 2).

The relative configuration of **5** was deduced from NOESY data. NOE correlations from H-1 β to H₃-20, from H-11 β to H₃-17 and H₃-20, and from H₃-17 to H-14 placed these protons on the same face of the molecule, while H-3, H-5, and H₃-18 had α -orientation as confirmed by the correlations from H-3 to H-1 α , H-5, and H₃-18 (Fig. 3). An X-ray crystallographic experiment confirmed the absolute configuration of **5** as depicted (Fig. 4). The Cu/K α radiation used for the X-ray diffraction allowed the assignment of the absolute configuration of all of the stereogenic centers in **5** as 3*S*, 5*R*, 10*S*, 13*R* and 14*S*.

Wentinoid F (**6**), was obtained as colorless oil, was assigned the molecular formula $C_{20}H_{30}O_3$ on the basis of HRESIMS data. The ^{13}C NMR spectrum of **6** exhibited signals similar to those presented in 11 β -hydroxy-7-oxopimar-8(9),15-dien (**11**),¹⁸ except for the methylene group at δ_C 35.9 (C-3) in ^{13}C NMR spectrum of **11** was replaced by an oxymethine unit at δ_C 76.1 (C-3) in that of **6**, indicating that **6** was C-3 hydroxylated derivative of **11**. The 2D NMR correlations supported this inference by the COSY correlations from H₂-2 to H₂-1 and H-3 and HMBC correlations from H₃-18 and H₃-19 to C-3.

The NOE correlations from H-1 α to H-3 and H-11, from H-5 to H-3 and H₃-18, from H-6 α to H₃-18, from H-11 to H-15, and from H-14 α to H-15 indicated the same orientation of these groups (Fig. 3), whereas correlations from H-6 β to H₃-20 revealed that these groups were on the other face. The absolute configuration of **6** was established by TDDFT-ECD calculations. The experimental ECD spectrum of **6** matched well with that calculated for (3*S*,5*R*,10*S*,11*S*,13*R*)-**6** (Fig. 6). The structure and

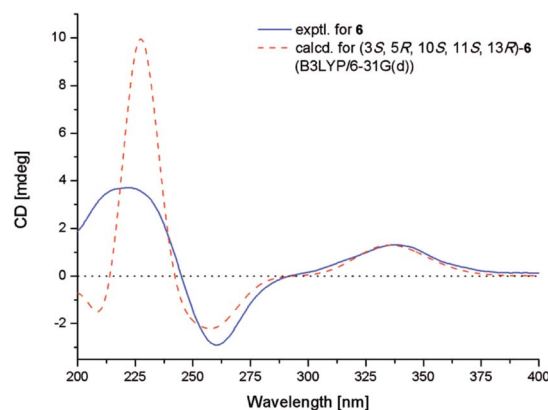
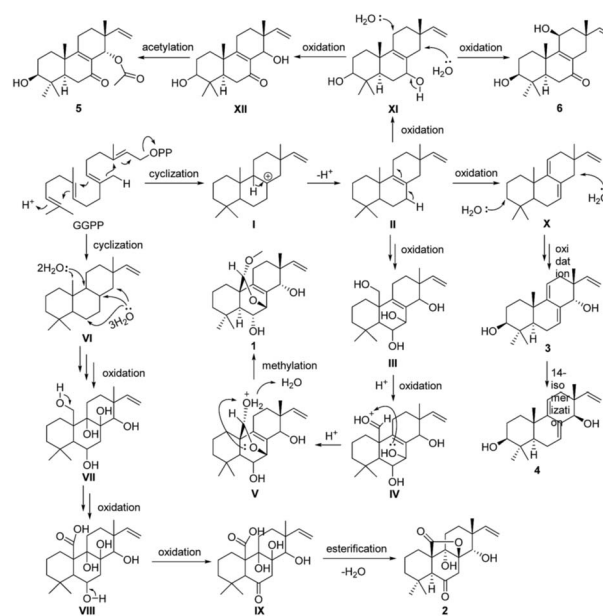


Fig. 6 Experimental and calculated ECD spectra of **6**.

absolute configuration of **6** were thus assigned as shown in Fig. 1.

A plausible biosynthetic pathway for compounds **1–6** is proposed as shown in Scheme 1. In this pathway, compounds **1–6** are produced from geranylgeranylpyrophosphate (GGPP). Intermediate **II**, produced by reduction of **I**, is presumed to be a common biosynthetic precursor of compounds **1** and **3–6**. After oxidation of **II** at C-7, C-14, and C-20, C-20 of intermediate **III** is further oxidized giving aldehyde derivative **IV**. Aldolization between the aldehyde group at C-20 and 7-OH in **IV** gives **V**, and successive methylation of 20-OH yields compound **1**. Deprotonation of H-7 and H-11 in **II** leads to **X**, which is further oxidized to give isomers **3** and **4**. Meanwhile, oxidation of C-3 and C-7 in **II** gives **XI**. Further oxidation of C-11 in **XI** yields **6**, whereas oxidation of C-14 in **XI** gives **5** via **XII**. After cyclization, GGPP is converted to **IX** via intermediates **VI–IX** by multistep oxidation of C-8, C-9, C-14, and C-20. Following esterification between carboxyl at C-20 and 8-OH, compound **2** is formed.



Scheme 1 Proposed biosynthetic pathway for compounds **1–6**.



The isolated compounds were tested against 11 human-, and aqua-pathogenic bacteria and seven plant-pathogenic fungi. Compound **1** exhibited selective activities against *Phytophthora parasitica*, *Fusarium oxysporum* f. sp. *lycopersici*, *Fusarium graminearum*, and *Botryosphaeria dothidea* with MIC values of 8.0, 4.0, 1.0, and 4.0 $\mu\text{g mL}^{-1}$, respectively, which were comparable to that of the positive control, amphotericin B (MIC = 2.0, 1.0, 1.0, and 2.0 $\mu\text{g mL}^{-1}$, respectively). However, other compounds didn't displayed potent inhibitory activity.

Conclusions

In summary, we isolated and characterized six new compounds wentinoids A–F (**1**–**6**), which are new members of highly oxygenated isopimarane-type diterpenoids. Among them, wentinoid A (**1**) contains unusual 20-acetal moiety and 7,20-oxa-bridged functionality, whereas wentinoid B (**2**) possesses a novel 8,20-lactone-bridged scaffold. A plausible biosynthesis mechanism for compounds **1**–**6** was proposed. The discovery of these compounds might provide further insight into the biosynthesis of isopimarane family and also provide new targets for synthetic or biosynthetic studies. Compound **1**, which may prove useful as antifungal agent, exhibited potent antimicrobial activities against some plant pathogenic fungi.

Experimental section

General experimental procedures

Melting points were determined with an SGW X-4 micromelting-point apparatus. Optical rotations were measured on an Optical Activity AA-55 polarimeter. UV spectra were measured on a PuXi TU-1810 UV-visible spectrophotometer. CD spectra were acquired on a JASCO J-715 spectropolarimeter. 1D and 2D NMR spectra were recorded at 500 and 125 MHz for ^1H and ^{13}C , respectively, on a Bruker Avance 500 MHz spectrometer with TMS as internal standard. Mass spectra were obtained on a VG Autospec 3000 or an API QSTAR Pulsar 1 mass spectrometer. Analytical and semi-preparative HPLC were performed using a Dionex HPLC system equipped with a P680 pump, an ASI-100 automated sample injector, and a UVD340U multiple wavelength detector controlled by Chromeleon software (version 6.80). Commercially available Si gel (200–300 mesh, Qingdao Haiyang Chemical Co.), Lobar LiChroprep RP-18 (40–63 μm , Merck), and Sephadex LH-20 (Pharmacia) were used for open column chromatography. All solvents were distilled prior to use.

Fungal material

The isolation and identification of the fungal material were identical to those described in our previous report.¹⁵ The strain is preserved at the Key Laboratory of Experimental Marine Biology, Institute of Oceanology of the Chinese Academy of Sciences (IOCAS), with accession number SD-310.

Cultivation

For chemical investigations, the fungal strain was dynamic fermented at a 500 L fermentator preloaded with 300 L of

sterilized liquid medium containing 50% (v/v) sea water (20% potato, 2% glucose, 0.5% peptone, and 0.3% yeast extract, pH 6.0) for 7 days at room temperature.

Extraction and isolation

The whole fermented cultures were filtered to separate the broth from the mycelia. The former was extracted three times with EtOAc, while the latter was extracted three times with a mixture of acetone and water (80 : 20, v/v). The acetone solution was evaporated under reduced pressure to afford an aqueous solution, which was then extracted three times with EtOAc. Since the TLC and HPLC profiles of the two EtOAc solutions from the broth and mycelia were almost identical, they were combined and concentrated under reduced pressure to give an extract (34.7 g) for further separation.

The combined extract was fractionated by silica gel vacuum liquid chromatography (VLC) using a stepwise gradient of a mixture of petroleum ether (PE)-ethyl acetate (EtOAc) (1 : 0, 50 : 1, 20 : 1, 5 : 1, 2 : 1 and 1 : 1) and CH_2Cl_2 -MeOH (20 : 1, 10 : 1, 5 : 1 and 0 : 1) to yield 10 major primary fractions (Fr.1–Fr.10). Fr.4 (7.1 g) was separated by CC on Lobar LiChroprep C_{18} eluting with MeOH– H_2O gradient to give nine subfractions (Frs. 4.1–4.9). Further purification of Fr. 4.4 by CC on silica gel with a CH_2Cl_2 -MeOH gradient (from 50 : 1 to 5 : 1) yielded Frs. 4.4.1–4.4.9, and then Fr. 4.4.3 was purified by CC on silica gel eluted with PE-EtOAc 5 : 1 to afford compounds **3** (10.9 mg) and **4** (8.3 mg). Fr. 4.5 was also resolved (Frs. 4.5.1–4.5.9) by CC on silica gel eluting with a CH_2Cl_2 -MeOH gradient (from 50 : 1 to 5 : 1). Fr. 4.5.5 was further subjected to CC on silica gel eluting with a PE-EtOAc gradient (from 5 : 1 to 2 : 1) to yield compounds **1** (14.4 mg) and **2** (8.7 mg). Fr. 4.6 was chromatographed over silica gel (CH_2Cl_2 -MeOH 50 : 1–15 : 1) to get Fr. 4.6.8, which was then purified by preparative TLC (PE-EtOAc 5 : 1) to give compound **5** (R_f = 0.4, 5.8 mg) and **6** (R_f = 0.6, 3.5 mg). Fr. 4.2 was subfractioned by CC on silica gel eluting with PE-EtOAc 15 : 1, then further purified by Sephadex LH-20 (MeOH) to yield **7** (6.6 mg).

Wentinoid A (1). Colorless oily powder. $[\alpha]_{25}^D$ –114 (c 0.22, MeOH). mp 125–127 °C. UV (MeOH) λ_{max} (log ϵ): 204 (2.07), 229 (1.63) nm. ECD (MeCN, λ [nm] ($\Delta\epsilon$), c = 1.1×10^{-3} M): 207 (–16.0). ^1H and ^{13}C -NMR: see Tables 1 and 2 ESIMS (positive): m/z 371 $[\text{M} + \text{Na}]^+$. HRESIMS (positive): m/z 371.2203 $[\text{M} + \text{Na}]^+$, calcd for $\text{C}_{21}\text{H}_{32}\text{O}_4\text{Na}$ 371.2193).

Wentinoid B (2). Colorless oil. $[\alpha]_{25}^D$ –21 (c 0.48, MeOH). UV (MeOH) λ_{max} (log ϵ): 209 (2.99), 270 (2.30) nm. ECD (MeCN, λ [nm] ($\Delta\epsilon$), c = 1.1×10^{-3} M): 228 (+2.9), 301 (–0.6), 348 (+0.2) nm. ^1H and ^{13}C -NMR: see Tables 1 and 2 ESIMS (positive): m/z 371 $[\text{M} + \text{Na}]^+$. HRESIMS (positive): m/z 371.1832 $[\text{M} + \text{Na}]^+$, calcd for $\text{C}_{20}\text{H}_{28}\text{O}_5\text{Na}$ 371.1829).

Wentinoid C (3). Colorless oily powder. $[\alpha]_{25}^D$ –63 (c 0.65, MeOH). mp 133–135 °C. UV (MeOH) λ_{max} (log ϵ): 238 (3.20) nm. ECD (MeCN, λ [nm] ($\Delta\epsilon$), c = 1.2×10^{-3} M): 234 (–24.6). ^1H and ^{13}C -NMR: see Tables 1 and 2 EIMS: m/z 302 $[\text{M}]^+$, 287 $[\text{M} - \text{CH}_3]^+$, 284 $[\text{M} - \text{H}_2\text{O}]^+$, 270 $[\text{M} - \text{CH}_2\text{O}]^+$, 266 $[\text{M} - 2\text{H}_2\text{O}]^+$. HREIMS: m/z 302.2248 $[\text{M}]^+$, calcd for $\text{C}_{20}\text{H}_{30}\text{O}_2$ 302.2246).



Wentinoid D (4). Colorless oil. $[\alpha]_{25}^D -11$ (c 0.46, MeOH). UV (MeOH) λ_{\max} (log ϵ): 240 (3.16) nm. ECD (MeCN, λ [nm] ($\Delta\epsilon$), $c = 1.2 \times 10^{-3}$ M): 238 (−24.6). ^1H and ^{13}C -NMR: see Tables 1 and 2 EIMS (negative): m/z 302 $[\text{M}]^+$, 287 $[\text{M} - \text{CH}_3]^+$, 284 $[\text{M} - \text{H}_2\text{O}]^+$, 270 $[\text{M} - \text{CH}_2\text{O}]^+$, 266 $[\text{M} - 2\text{H}_2\text{O}]^+$. HREIMS (positive): m/z 302.2242 ($[\text{M}]^+$, calcd. for $\text{C}_{20}\text{H}_{30}\text{O}_2$ 302.2246).

Wentinoid E (5). Colorless oily powder. $[\alpha]_{25}^D -30$ (c 0.46, MeOH). mp 137–139 °C. UV (MeOH) λ_{\max} (log ϵ): 242 (3.31) nm. ECD (MeCN, λ [nm] ($\Delta\epsilon$), $c = 1.0 \times 10^{-3}$ M): 212 (−23.2), 242 (−57.2), 332 (+6.0) nm. ^1H and ^{13}C -NMR: see Tables 1 and 2 ESIMS (positive): m/z 361 $[\text{M} + \text{H}]^+$. HRESIMS (positive): m/z 361.2367 ($[\text{M} + \text{H}]^+$, calcd. for $\text{C}_{22}\text{H}_{33}\text{O}_4$ 361.2373).

Wentinoid F (6). Colorless oil. $[\alpha]_{25}^D +82$ (c 0.17, MeOH). UV (MeOH) λ_{\max} (log ϵ): 250 (2.05) nm. ECD (MeCN, λ [nm] ($\Delta\epsilon$), $c = 1.2 \times 10^{-3}$ M): 219 (+1.9), 261 (−1.6), 340 (+0.7) nm. ^1H and ^{13}C -NMR: see Tables 1 and 2 ESIMS (positive): m/z 341 $[\text{M} + \text{Na}]^+$. HRESIMS (positive): m/z 341.2082 ($[\text{M} + \text{Na}]^+$, calcd. for $\text{C}_{20}\text{H}_{30}\text{O}_3\text{Na}$ 341.2087).

Computational section

Conformational searches were performed *via* the molecular mechanics using MM + method in HyperChem 8.0 software, and the geometries were further optimized at B3LYP/6-31G(d) level *in vacuo* *via* Gaussian 09 software to give the energy-minimized conformers. Then, the optimized conformers were subjected to the calculations of ECD spectra using TD-DFT at B3LYP/6-31G(d) level; solvent effects of the MeCN solution were evaluated at the same DFT level using the SCRF/PCM method.¹⁹

X-ray crystallographic analysis of compounds 1, 3 and 5

All crystallographic data were collected on a Bruker Smart-1000 CCD diffractometer using graphite-monochromated Cu-K α radiation ($\lambda = 1.54178$ Å) at 293(2) K. The data were corrected for absorption using the program SADABS.²⁰ The structures were solved by direct methods using the SHELXTL software package.²¹ All non-hydrogen atoms were refined anisotropically. The H atoms were located by geometrical calculations, and their positions and thermal parameters were fixed during the structure refinement. The structures were refined using full-matrix least-squares techniques.²²

Crystal data for compound 1. $\text{C}_{21}\text{H}_{32}\text{O}_4$; F.W. = 348.47; orthorhombic space group $P2_12_12_1$; unit cell dimensions $a = 8.8580(6)$ Å, $b = 12.4336(7)$ Å, $c = 17.5420(12)$ Å, $V = 1932.0(2)$ Å³, $\alpha = \beta = \gamma = 90^\circ$; $Z = 4$; $d_{\text{calcd}} = 1.198$ mg m^{−3}; crystal dimensions $0.40 \times 0.37 \times 0.30$ mm; $\mu = 0.647$ mm^{−1}; and $F(000) = 760$. The 2860 measurements yielded 1907 independent reflections after equivalent data were averaged and Lorentz and polarization corrections were applied. The final refinement provided $R_1 = 0.0894$ and $wR_2 = 0.1289$ [$I > 2\sigma(I)$]. The Flack parameter was 0.0(5) in the final refinement for all 2860 reflections, with 1907 Friedel pairs.

Crystal data for compound 3. $\text{C}_{20}\text{H}_{30}\text{O}_2 \cdot \text{H}_2\text{O}$; F.W. = 320.46; orthorhombic space group $P2_12_12_1$; unit cell dimensions $a = 6.6729(5)$ Å, $b = 11.9119(9)$ Å, $c = 23.5741(18)$ Å, $V = 1873.8(2)$ Å³, $\alpha = \beta = \gamma = 90^\circ$; $Z = 4$; $d_{\text{calcd}} = 1.136$ mg m^{−3}; crystal dimensions $0.23 \times 0.18 \times 0.07$ mm; $\mu = 0.583$ mm^{−1}; and

$F(000) = 704$. The 3169 measurements yielded 1091 independent reflections after equivalent data were averaged and Lorentz and polarization corrections were applied. The final refinement provided $R_1 = 0.2224$ and $wR_2 = 0.2979$ [$I > 2\sigma(I)$]. The Flack parameter was 0.0(14) in the final refinement for all 3169 reflections, with 1091 Friedel pairs.

Crystal data for compound 5. $\text{C}_{22}\text{H}_{32}\text{O}_4$; F.W. = 360.48; orthorhombic space group $P2_12_12_1$; unit cell dimensions $a = 9.4327(6)$ Å, $b = 13.1356(9)$ Å, $c = 16.7370(9)$ Å, $V = 2073.8(2)$ Å³, $\alpha = \beta = \gamma = 90^\circ$; $Z = 4$; $d_{\text{calcd}} = 1.155$ mg m^{−3}; crystal dimensions $0.45 \times 0.25 \times 0.20$ mm; $\mu = 0.620$ mm^{−1}; and $F(000) = 784$. The 2922 measurements yielded 2104 independent reflections after equivalent data were averaged and Lorentz and polarization corrections were applied. The final refinement provided $R_1 = 0.0722$ and $wR_2 = 0.1211$ [$I > 2\sigma(I)$]. The Flack parameter was 0.0(4) in the final refinement for all 2922 reflections, with 2104 Friedel pairs.

Antimicrobial assay

Antimicrobial assay against eleven human-, and aqua-pathogenic bacteria *P. aeruginosa*, *E. tarda*, *A. hydrophilia*, *M. luteus*, *E. tarda*, *E. coli*, *V. parahaemolyticus*, *S. aureus*, *V. alginolyticus*, *V. harveyi*, and *E. tarda* as well as seven plant pathogenic fungi *C. albicans*, *P. parasitica*, *C. nicotianae*, *A. alternata*, *F. oxysporum* f. sp. *lycopersici*, *F. Graminearum*, and *B. dothidea* was carried out using the well diffusion method.²³ Chloramphenicol and Amphotericin B were used as a positive control for the bacteria and fungi, respectively.

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