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Terretonin, ophiobolin, and drimane terpenes with absolute configurations from an algicolous *Aspergillus ustus*†

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One new meroterpene, 1,2-dihydroterretonin F (**2**), five new sesterterpenes, (6α)-21-deoxyophiobolin G (**3**), (6α)-16,17-dihydro-21-deoxyophiobolin G (**4**), ophiobolin U (**5**), ophiobolin V (**6**), and ophiobolin W (**7**), two new sesquiterpenes, (6-strobilactone-B) esters of (*E*,*E*)-6,7-epoxy-2,4-octadienoic acids (**13** and **14**), and twelve known terpenes (**1**, **8**–**12**, and **15**–**20**) were isolated from *Aspergillus ustus*, a fungus from the fresh tissue of marine green alga *Codium fragile*. Their structures and absolute configurations were identified by NMR and mass spectroscopic methods as well as quantum chemical calculations. Some of the isolates exhibited antibacterial activity and brine shrimp toxicity.

Introduction

Terpenes are produced biogenetically through mevalonate pathways, which comprise hemi-, mono-, sesqui-, di-, sester-, tri-, sesquart-, and tetraterpenes and represent the largest group of natural products. They exhibit various bioactivities, including antibacterial, antifungal, antiinsectan, cytotoxicity, *etc.*, but not much is known about their true biological and ecological functions.[1,2] *Aspergillus ustus* (*A. insuetus*) has been proven to be a prolific producer of terpenes, containing sesquiterpenes, sesterterpenes, and meroterpenes.[3–7] However, most of them have been assigned only relative configurations, and their absolute configurations remain unresolved. In our ongoing program to discover new bioactive compounds from marine algae and their associated fungi, a fungus *A. ustus* isolated from the marine green alga *Codium fragile* was investigated. As a result, one new meroterpene, 1,2-dihydroterretonin F (**2**), five new sesterterpenes, (6α)-21-deoxyophiobolin G (**3**), (6α)-16,17-dihydro-21-deoxyophiobolin G (**4**), ophiobolin U (**5**), ophiobolin V (**6**), and ophiobolin W (**7**), two new sesquiterpenes, (6-strobilactone-B) esters of (*E*,*E*)-6,7-epoxy-2,4-octadienoic acids (**13** and **14**), and twelve known terpenes, terretonin F (**1**, revised),[6] (6α)-21,21-*O*-dihydroophiobolin G (**8**),[3] (5α,6α)-ophiobolin H (**9**),[3] ophiobolin H (**10**),[8] ophiobolin F (**11**),[9] 3β,9α,11-trihydroxy-6-oxodrim-7-ene (**12**),[4] (6-strobilactone-B) esters of (*E*,*E*)-6,7-dihydroxy-2,4-octadienoic acids (**15** and **16**),[4] (6-strobilactone-B) ester of (*E*,*E*)-6-oxo-2,4-hexadienoic acid (**17**, Ustusolate E),[4,5] Ustusolate D (**18**),[5] 9α-hydroxy-6β-[(2*E*,4*E*,6*E*)-octa-2,4,6-trienoyloxy]-5α-drim-7-en-11,12-olide (**19**),[10] and (2′*E*,4′*E*,6′*E*)-6-(1′-carboxyocta-2′,4′,6′-triene)-11,12-epoxy-9,11-dihydroxydrim-7-ene (**20**),[10] were isolated and identified (Fig. 1). Details of the isolation, structure elucidation, and bioactivity of these compounds are presented here.

Results and discussion

Compound **1** was obtained as a white powder. The ^{1}H NMR spectrum along with HSQC data exhibited five methyl singlets at δ_H 1.27 (s, H-21), 1.54 (s, H-18), 1.58 (s, H-19), 1.82 (s, H-20), and 1.87 (s, H-23), one methyl doublet at δ_H 1.57 (d, 6.7 Hz, H-25), one methoxyl singlet at δ_H 3.82 (s, H-1′), one quartet at δ_H 4.78 (q, 6.7 Hz, H-16) ascribable to an oxymethine, a pair of doublets at δ_H 4.93 (d, 0.9 Hz, H-22a) and 5.10 (d, 1.7 Hz, H-22b) attributable to an exocyclic methylene, two mutually coupled olefinic protons at δ_H 6.11 (d, 10 Hz, H-2) and 6.80 (d, 10 Hz, H-1), and one exchangeable proton at δ_H 6.68 (s, OH-6). The ^{13}C and DEPT NMR spectra showed 26 resonances, corresponding to seven methyls, two methylenes, four methines, and thirteen unprotonated carbons. The above ^{1}H and ^{13}C NMR data were the same as those reported for terretonin F, which revealed that **1** should possess the same structure and relative configuration as terretonin F.[6] However, the obvious HMBC correlations from H-23 to C-12, C-13, C-14, and C-17 (δ_C 169.8) and from H-25 to C-15 (δ_C 204.1) and C-16 and no correlation from H-23 to downfield C-15 of **1** and terretonin F (ESI†) suggested that an oxygen atom should be bonded to C-17 to form a lactone and an oxygenated ethyl group was attached to C-15 (Fig. 1). The other ^{1}H–^{1}H COSY and

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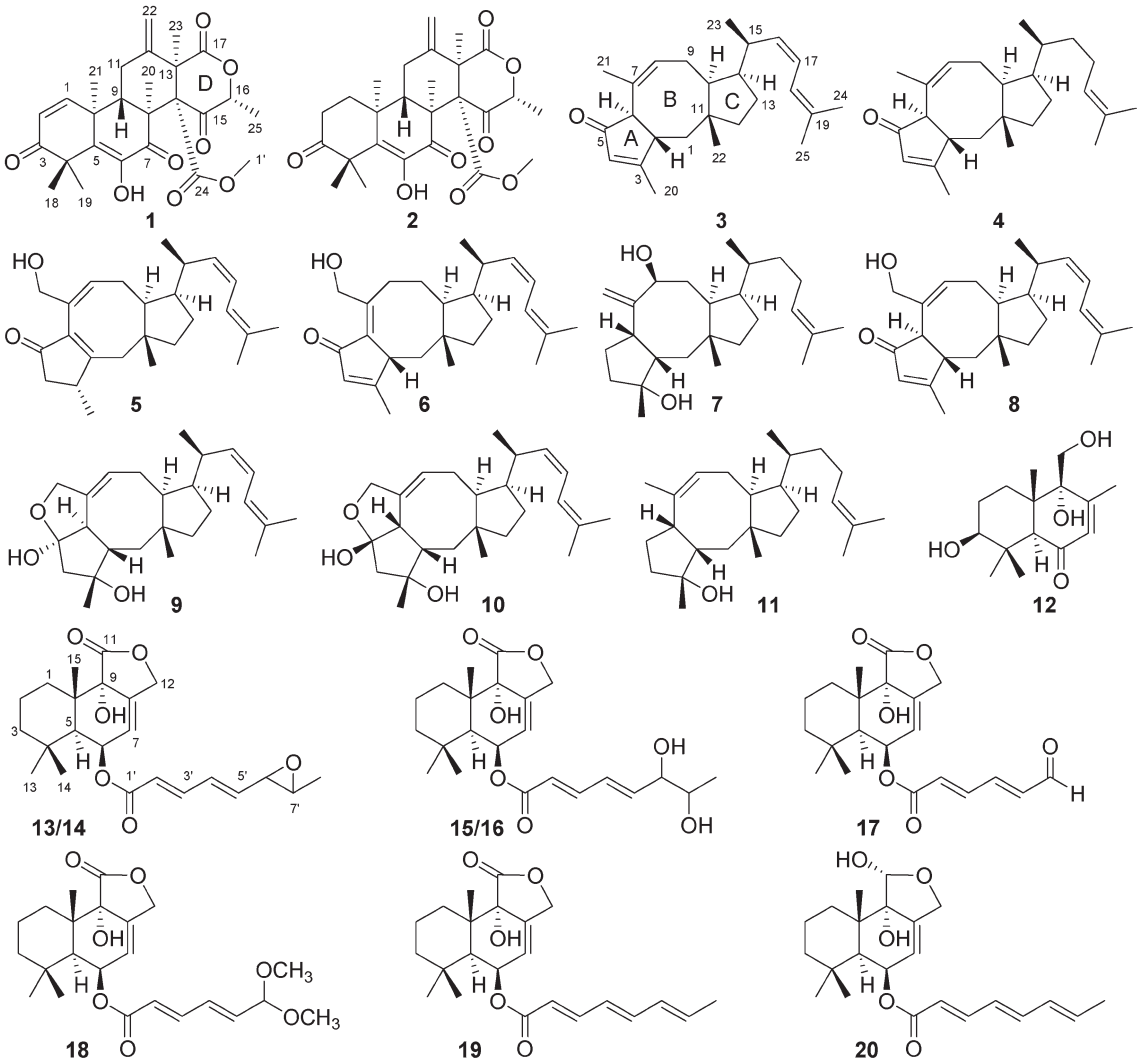

Fig. 1 Structures of compounds **1–20**.

HMBC correlations (Fig. 2) further confirmed the structure of **1**. The NOE correlations of H-1′ with H-20, H-23, and H-25 and H-9 with H-16 indicated a *syn* orientation of C-20, C-23, C-25, and the carbomethoxy group (C-24), which was not assigned originally.[6] On the other hand, the assignment of ring D in terretonin E was also deduced to be the same as that of **1** according to its original HMBC correlations from H-23 (δ_H 1.86) to C-12 (δ_C 142.1), C-13 (δ_C 54.8), C-14 (δ_C 65.9), and C-17 (δ_C 170.0).

Compound **2** was obtained as a colorless oil. A molecular formula of $C_{26}H_{32}O_8$ was determined by HREIMS (*m*/*z* 472.2093 $[M]^+$, calcd for $C_{26}H_{32}O_8$, 472.2097), requiring eleven degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) displayed five methyl singlets, one methyl doublet, one methoxyl singlet, one quartet ascribed to an oxymethine, a pair of doublets attributed to an exocyclic methylene, and one hydroxyl proton. The ^{13}C and DEPT NMR spectra (Table 2) demonstrated the presence of seven methyls, four methylenes, two methines, and thirteen quaternary carbons. The above NMR data closely resembled that of **1** except for the presence of signals for two methylenes and the lack of signals for two mutually coupled olefinic methines. Hence, **2** was deduced to be a hydrogenated derivative of **1** at C-1 and C-2. This proposal was further supported by ^{1}H–^{1}H COSY and HMBC correlations as illustrated in Fig. 2.

Quantum chemical calculations of electronic circular dichroism (ECD) spectra have been proven to be reliable tools in deducing the absolute configurations of natural products.[11] To establish the absolute configuration of **2**, its ECD spectrum was determined and calculated. The energy-minimized conformer was generated by the Dreiding force field,[12] which was subjected to the theoretical calculation of ECD spectrum using the time-dependent density function theory (TD-DFT) method at the B3LYP/6-31G(d) level after optimization at the B3LYP/6-31G(d) level in methanol (Fig. 3). The calculated ECD spectrum produced by SpecDis software was in good accordance with the experimental one (Fig. 4),[13] which suggested the absolute configuration of **2** to be 8*S*, 9*S*, 10*S*, 13*R*, 14*R*, and 16*R*. Then,

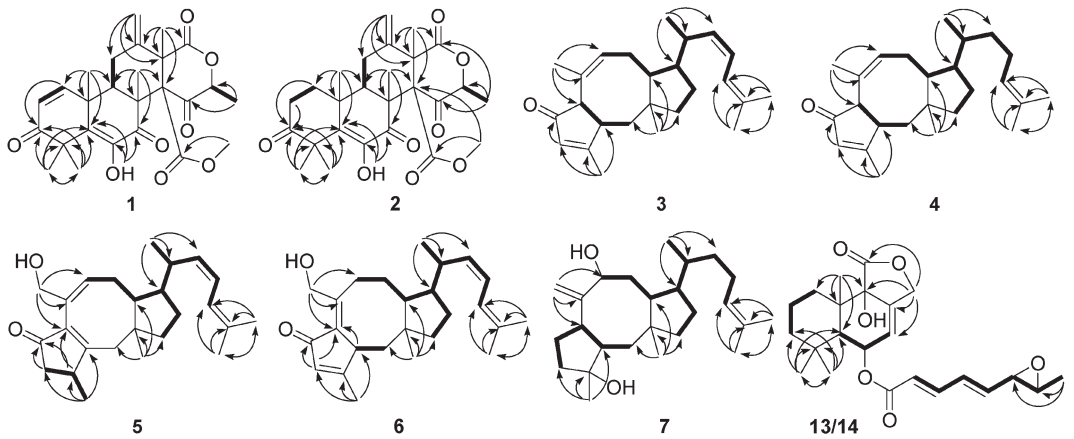

Fig. 2 Key ^{1}H-^{1}H COSY (solid line) and HMBC (arrow) correlations of **1**–**7**,**13**,**14**.

Table 1 ^{1}H NMR data for **2–7** in $CDCl_3$

Position	2	3	4	5	6	7
1a	1.79, dd (13.5, 10.4)	1.12, dd (13.0, 12.8)	1.13, dd (13.0, 12.8)	2.08, d (12.4)	0.98, dd (12.9, 11.9)	1.20, dd (12.6, 11.7)
1b	2.11, dd (13.5, 8.7)	2.02, dd (13.0, 3.6)	2.02, dd (13.0, 3.6)	2.59, d (12.4)	1.98, d (12.9)	1.39, dd, (12.6, 1.5)
2a	2.53, dd (19.1, 10.4)	2.67, brd (12.8)	2.69, brd (12.8)	—	3.21, brd (11.9)	1.87, ddd (11.7, 10.5, 1.5)
2b	2.70, dd (19.1, 8.7)	—	—	—	—	—
3	—	—	—	2.94, qd (7.2, 6.4)	—	—
4a	—	5.89, brs	5.90, brs	2.12, d (18.8)	6.04, brs	1.57, m
4b	—	—	—	2.76, dd (18.8, 6.4)	—	1.81, m
5a	—	—	—	—	—	1.60, m
5b	—	—	—	—	—	1.94, m
6	—	3.54, d (3.3)	3.59, d (3.4)	—	—	2.99, ddd (10.5, 9.3, 9.3)
8a	—	5.39, brd (6.3)	5.44, brd (6.0)	6.14, dd (8.0, 8.0)	2.13, m	4.05, dd (11.1, 6.0)
8b	—	—	—	—	2.59, dddd (12.8, 12.8, 12.8, 6.9)	—
9a	1.99, dd (13.7, 3.0)	1.78, m	1.81, m	1.68, m	1.40, m	1.45, m
9b	—	2.49, brd (19.4)	2.33, brd (18.6)	2.62, dd (13.0, 8.0)	1.58, m	1.79, m
10	—	2.55, m	2.62, ddd (14.6, 10.2, 3.8)	1.59, m	1.99, m	2.29, m
11a	2.35, dd (14.2, 3.0)	—	—	—	—	—
11b	2.55, ddd (14.2, 13.7, 1.7)	—	—	—	—	—
12a	—	1.40, ddd (12.2, 11.9, 5.2)	1.36, m	1.37, m	1.30, m	1.31, m
12b	—	1.49, ddd (11.9, 5.2, 1.2)	1.47, m	1.49, dd (11.8, 7.4)	1.45, m	1.34, m
13a	—	1.27, dddd (12.2, 12.1, 11.4, 5.2)	1.23, dddd (12.3, 12.3, 11.6, 5.1)	1.67, m	1.54, m	1.39, m
13b	—	1.62, brdd (11.4, 5.2)	1.52, brdd (11.6, 6.0)	1.90, m	1.99, m	1.46, m
14	—	1.85, m	1.71, m	2.05, m	1.91, m	1.36, m
15	—	2.54, m	1.39, m	2.58, m	2.59, m	1.92, m
16a	4.78, q (6.7)	5.11, dd (10.9, 9.7)	0.93, m	5.18, dd (11.2, 10.2)	5.19, dd (9.3, 9.3)	1.15, m
16b	—	—	1.43, m	—	—	1.23, m
17a	—	6.04, dd (11.6, 10.9)	1.89, m	5.99, dd (11.6, 11.2)	6.02, dd (12.2, 9.3)	1.97, m
17b	—	—	2.04, m	—	—	1.97, m
18	1.46, s	5.99, d (11.6)	5.11, t (7.2)	5.90, d (11.6)	6.02, d (12.2)	5.11, brt (7.2)
19	1.54, s	—	—	—	—	—
20	1.79, s	2.08, s	2.08, s	1.22, d (7.2)	2.10, s	1.25, s
21a	1.03, s	1.56, brs	1.60, brs	4.00, m	4.11, dd (15.8, 10.1)	5.20, d (1.4)
21b	—	—	—	4.00, m	4.45, dd (15.8, 3.8)	5.24, d (1.4)
22a	4.89, d (1.0)	1.00, s	0.99, s	0.90, s	1.13, s	0.74, s
22b	5.04, d (1.7)	—	—	—	—	—
23	1.86, s	0.93, d (6.7)	0.86, d (6.6)	0.87, d (6.8)	0.91, d (6.8)	0.71, d (6.8)
24	—	1.75, s	1.60, s	1.72, s	1.75, s	1.61, s
25	1.58, d (6.7)	1.81, s	1.69, s	1.78, s	1.82, s	1.69, s
1′	3.82, s	—	—	—	—	—
OH	6.55, s	—	—	4.00, m	5.71, dd (10.1, 3.8)	—

Table 2 ^{13}C NMR data for **2–7** in $CDCl_3$

Position	2	3	4	5	6	7
1	34.2, CH_2	46.3, CH_2	46.3, CH_2	40.8, CH_2	49.3, CH_2	38.1, CH_2
2	32.6, CH_2	49.2, CH	49.1, CH	181.6, C	46.9, CH	47.0, CH
3	214.0, C	181.0, C	181.0, C	39.1, CH	176.7, C	81.9, C
4	48.0, C	130.5, CH	130.5, CH	43.6, CH_2	131.7, CH	41.3, CH_2
5	137.2, C	209.3, C	209.2, C	210.2, C	197.8, C	32.7, CH_2
6	139.1, C	54.2, CH	54.2, CH	140.4, C	138.6, C	37.3, CH
7	195.6, C	128.6, C	129.1, C	132.4, C	152.9, C	152.6, C
8	49.3, C	129.3, CH	128.8, CH	137.8, CH	33.3, CH_2	79.8, CH
9	46.3, CH	29.0, CH_2	29.2, CH_2	23.5, CH_2	28.3, CH_2	28.9, CH_2
10	37.9, C	44.0, CH	43.3, CH	52.1, CH	43.9, CH	45.1, CH
11	28.2, CH_2	45.1, C	44.9, C	41.1, C	43.8, C	43.5, C
12	143.7, C	44.4, CH_2	44.7, CH_2	40.7, CH_2	45.0, CH_2	42.9, CH_2
13	54.8, C	27.8, CH_2	27.1, CH_2	27.6, CH_2	25.2, CH_2	22.9, CH_2
14	65.8, C	52.2, CH	51.5, CH	45.4, CH	50.5, CH	45.0, CH
15	204.2, C	32.2, CH	31.7, CH	35.3, CH	33.0, CH	32.8, CH
16	78.8, CH	136.6, CH	37.5, CH_2	137.3, CH	137.8, CH	36.9, CH_2
17	170.0, C	123.3, CH	25.9, CH_2	122.2, CH	122.3, CH	26.2, CH_2
18	20.4, CH_3	120.4, CH	124.7, CH	120.1, CH	120.5, CH	124.9, CH
19	24.4, CH_3	135.5, C	131.2, C	135.6, C	135.1, C	131.2, C
20	19.2, CH_3	17.4, CH_3	17.4, CH_3	19.3, CH_3	17.4, CH_3	26.6, CH_3
21	16.9, CH_3	21.3, CH_3	21.5, CH_3	66.8, CH_2	63.8, CH_2	117.7, CH_2
22	113.0, CH_2	22.6, CH_3	22.7, CH_3	19.4, CH_3	21.7, CH_3	19.8, CH_3
23	21.7, CH_3	21.2, CH_3	18.6, CH_3	20.2, CH_3	20.8, CH_3	15.9, CH_3
24	167.1, C	18.2, CH_3	17.7, CH_3	16.6, CH_3	18.2, CH_3	17.6, CH_3
25	17.9, CH_3	26.5, CH_3	25.7, CH_3	26.5, CH_3	26.4, CH_3	25.8, CH_3
1′	53.2, CH_3	—	—	—	—	—

the absolute configuration of **1** was also deduced to be 8*S*, 9*S*, 10*S*, 13*R*, 14*R*, and 16*R* based on biogenic considerations and their similar specific optical rotations (−19 reported for **1**).[6]

Compound **3** was obtained as a colorless oil and assigned a molecular formula $C_{25}H_{36}O$ by HREIMS, which implied eight degrees of unsaturation. The presence of a conjugated carbonyl group was indicated by the IR absorption at 1697 cm^{-1}. The ^1H NMR spectrum (Table 1) showed five methyl singlets, one methyl doublet, and five signals of olefinic protons. The ^{13}C NMR spectrum (Table 2) exhibited 25 resonances, which were classified into six methyls, four methylenes, ten methines, and five unprotonated carbons by DEPT and HSQC experiments. The above NMR data were similar to those reported for (6α)-21,21-*O*-dihydroophiobolin G (**8**) except for the presence of an additional olefinic methyl and the lack of a hydroxylated methylene.[3] Thus, **3** was deduced to be a C-21 deoxy derivative of **8**, which was confirmed by ^1H–^1H COSY and HMBC correlations (Fig. 2). The relative configuration of **3** was determined by a NOESY experiment. The NOE correlations between H-1a/H-10, H-1a/H-6, H-10/H-14 suggested a *syn* orientation of them, and H-1b, H-2, and C-22 were oriented on the opposite face relative to the above protons based on NOE correlations between H-2/H-1b, H-2/H-22. The relative configurations at C-10, C-14, and C-15 were supported by the identical NMR data with those reported for 6-*epi*-ophiobolin G.[8] Additionally, the double bond at C-16 was deduced to be *cis* according to the coupling constant (J = 10.9 Hz) between H-16/H-17. The above data evidenced the structure of **3**, trivially named (6α)-21-deoxyophiobolin G.

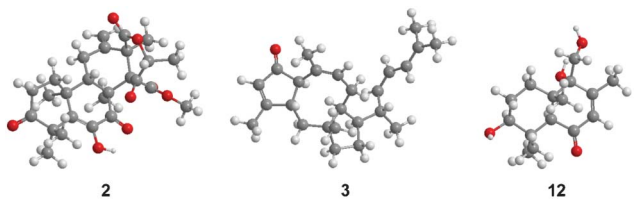

Fig. 3 Energy-minimized conformers of **2**, **3**, and **12** (in MeOH).

Compound **4** was obtained as a colorless oil with a molecular formula of $C_{25}H_{38}O$ established by HREIMS. Its similar NMR data (Tables 1 and 2) with that of **3** revealed that **4** was an analogue of **3**. Two methylene signals at δ_C 37.5 (C-16) and 25.9 (C-17) appeared in the ^{13}C NMR spectrum of **4**, instead of the signals for two olefinic methine (C-16 and C-17) in that of **3**. Thus, **4** was deduced to be a hydrogenated derivative of **3** at C-16 and C-17. The structure was further supported by ^1H–^1H COSY and HMBC spectra, which provided the expected correlations (Fig. 2) to the new methylene protons and carbons. Compound **4** was trivially named (6α)-16,17-dihydro-21-deoxyophiobolin G.

Compound **5** was obtained as a colorless oil. A molecular formula of $C_{25}H_{36}O_2$ was assigned by HRESIMS. Examination of the NMR data for **5** (Tables 1 and 2) revealed that it primarily differed from **8** in the vicinity of ring A.[3] Based on HMBC (H-3 to C-5 and C-6; H-20 to C-2, C-3, and C-4; and H-21 to C-6, C-7, and C-8) and ^1H-^1H COSY (H-3 with H-4 and H-20) correlations (Fig. 2), the double bond at C-3 in **8** was found to be no longer present and instead a new double bond appeared between C-2/C-6 in **5**. Additionally, H-3 was determined to be

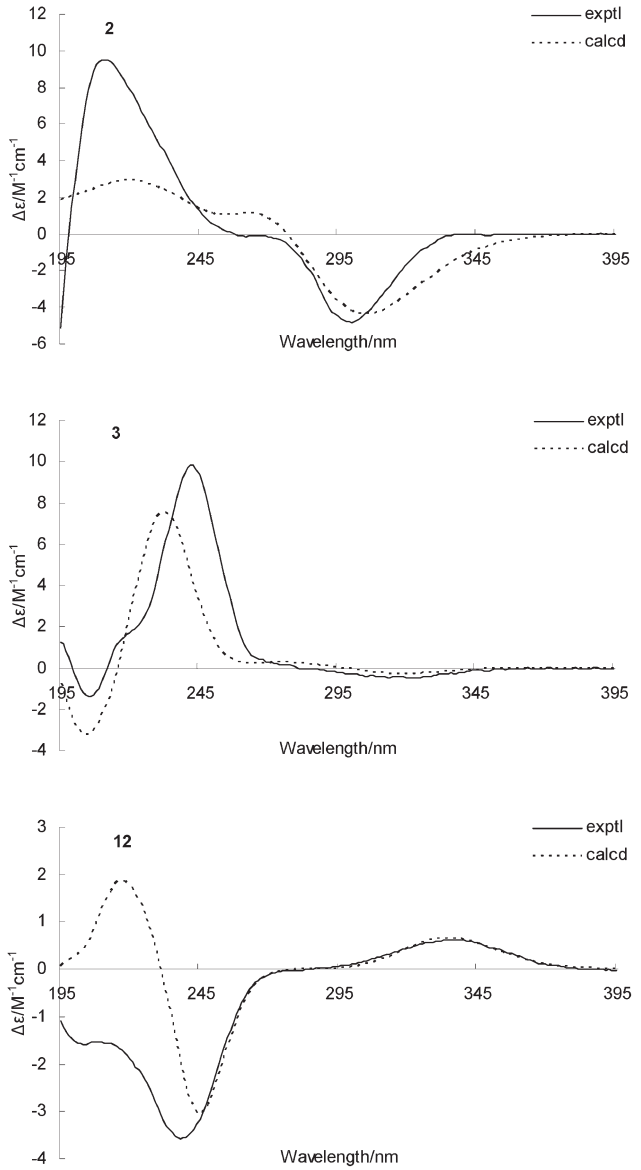

Fig. 4 Experimental and calculated ECD spectra of **2**, **3**, and **12**.

syn to C-22 by an NOE correlation between H-3 and H-22. Compound **5** was trivially named ophiobolin U.

Compound **6** was obtained as a colorless oil. A molecular formula of $C_{25}H_{36}O_2$ was established by HREIMS. A detailed NMR data (Tables 1 and 2) comparison with those reported for **8** revealed that they shared many structural similarities.[3] However, the double bond at C-7 in **8** was deduced to move to C-6 in **6** based on HMBC correlations from H-2 and H-4 to C-6 and from H-21 to C-6, C-7, and C-8 (methylene). The relative configuration at C-2 was deduced to be unaltered compared to that in **8** based on an NOE correlation between H-2 and H-22. The remaining 2D NMR correlations (Fig. 2) further supported the structure of **6**, trivially named ophiobolin V.

Compound **7** was obtained as a colorless oil. A molecular formula of $C_{25}H_{42}O_2$ was determined by HREIMS, corresponding to five degrees of unsaturation. Compared to **3–6**, compound **7** lacked a carbonyl group due to no IR absorptions in the region of 1660–1700 cm^{-1}. However, the ^1H and ^{13}C NMR data (Tables 1 and 2) were consistent with a structure featuring an ophiobolin backbone. The lipophilic side chain attached to ring-C was deduced to be the same as that of **4** based on their identical NMR data and HMBC correlations from H-23 to C-14, C-15, and C-16 and from H-24 and H-25 to C-18 and C-19. A proton spin system consisting of CH_2-1, CH-2, CH-6, CH_2-5, and CH_2-4 confirmed that the carbonyl group in ring A of **3–6** was missing and was replaced by a methylene group in **7**. Additionally, C-3 was hydroxylated based on its downfield shift (δ_C 81.9) and HMBC correlations from H-20 to C-2, C-3, and C-4, and the assignments at C-7, C-8, and C-21 was achieved by HMBC correlations from H-21 to C-6, C-7, and hydroxylated C-8 (δ_C 79.8). The cofacial orientation of H-2, H-6, C-20, and C-22 was established by NOE correlations. Furthermore, H-8 was deduced to be opposite to H-6 based on the lack of a correlation between them and the presence of a prominent NOE correlation between H-8 and Ha-5.

In order to confirm the absolute configurations of compounds **3–7**, the ECD spectrum of **3** was calculated, which matched well with the experimental one (Fig. 4). Then, the absolute configuration of **3** was suggested to be 2*S*, 6*R*, 10*S*, 11*R*, 14*R*, and 15*S*, and the absolute configurations of **4–7** were deduced to be 2*S*, 6*R*, 10*S*, 11*R*, 14*R*, and 15*S* (**4**), 3*R*, 10*S*, 11*R*, 14*R*, and 15*S* (**5**), 2*R*, 10*S*, 11*R*, 14*R*, and 15*S* (**6**), and 2*S*, 3*R*, 6*S*, 8*S*, 10*S*, 11*R*, 14*R*, and 15*S* (**7**), respectively, based on biogenic considerations and their identical sign (+) of specific optical rotations.

Compounds **13** and **14** were isolated as an oily mixture. A molecular formula of $C_{23}H_{30}O_6$ was assigned to both of them based on HRESIMS. They showed almost overlapping ^1H and ^{13}C NMR signals, and an examination of them revealed that **13** and **14** were different from **15–19** mainly at the end of fatty acyl moieties.[4,5,10] An epoxy group indicated by the characteristic signals at δ_H 2.96 (qd, 5.1, 2.0 Hz, H-7′) and 3.14 (brd, 7.5 Hz, H-6′) and δ_C 57.4 (C-7′) and 58.3/58.4 (C-6′) in **13**/**14** was assigned between C-6′ and C-7′ by HMBC correlations from H-8′ to C-6′ and C-7′ and ^1H-^1H COSY correlations between H-5′/H-6′, H-6′/H-7′, H-7′/H-8′.[14] The *anti* orientation of two epoxy protons was positioned by NOE correlations between H-5′/H-7′, H-6′/H-8′, and the relative configuration of the remaining moiety in **13**/**14** was deduced to be the same as that of **15**/**16** by their identical NMR data and biogenic considerations. Additionally, **13** and **14** were deduced to be diastereomers at C-6′ and C-7′ based on their slight differences of NMR data around these positions. The other 2D NMR correlations (Fig. 2) further confirmed the structures of **13** and **14** to be (6-Strobilactone-B) esters of (*E*,*E*)-6,7-epoxy-2,4-octadienoic acids.

The remaining known compounds, including (6α)-21,21-*O*-dihydroophiobolin G (**8**),[3] (5α,6α)-ophiobolin H (**9**),[3] ophiobolin H (**10**),[8] ophiobolin F (**11**),[9] 3β,9α,11-trihydroxy-6-oxodrim-7-ene (**12**),[4] (6-strobilactone-B) esters of (*E*,*E*)-6,7-dihydroxy-2,4-octadienoic acids (**15** and **16**),[4] (6-strobilactone-B) ester of (*E*,*E*)-6-oxo-2,4-hexadienoic acid (**17**, Ustusolate

E),[4,5] Ustusolate D (**18**),[5] 9α-hydroxy-6β-[(2*E*,4*E*,6*E*)-octa-2,4,6-trienoyloxy]-5α-drim-7-en-11,12-olide (**19**),[10] and (2′*E*,4′*E*,6′*E*)-6-(1′-carboxyocta-2′,4′,6′-triene)-11,12-epoxy-9,11-dihydroxy-drim-7-ene (**20**),[10] were identified by detailed NMR data comparison with literature values.

To establish the absolute configuration of sesquiterpene core in **13–20**, the ECD spectrum of **12** (a possible precursor) was calculated, which agreed well with the experimental one (Fig. 4). Thus, the absolute configuration of **12** was suggested to be 3*S*, 5*S*, 9*R*, and 10*S*, and the absolute configuration of sesquiterpene core in **13–20** was tentatively deduced to be 5*S*, 6*R*, 9*S*, and 10*S* (11*R* in **20**) based on biogenic considerations.

Compounds **1–20** were assayed for antibacterial and antifungal activities as well as brine shrimp toxicity.[15] The results showed that **5** and **9** exhibited inhibitory activities against *Escherichia coli* (inhibitory diameters of 15 and 10 mm, respectively), and **5** also showed activity against *Staphylococcus aureus* (10 mm) at 30 μg disk^{-1}. Additionally, **1**, **2**, **5**, **9**, **13**/**14**, and **17** displayed >75% lethality at 100 μg mL^{-1} and LC_{50} values of 23.3, 65.4, 48.1, 41.8, 62.2, and 48.9 μg mL^{-1}, respectively, in the brine shrimp (*Artemia salina*) toxicity assay. However, no compounds showed inhibitory activities against phytopathogenic fungi (*Colletotrichum lagenarium* and *Fusarium oxysporum*) at 30 μg disk^{-1}.

Conclusions

Overall, twenty terpenes, including eight new ones, with terretonin, ophiobolin, and drimane skeletons were isolated and identified from an algicolous *A. ustus* strain, which contributed greatly to the molecular diversity of this species. On the other hand, the absolute configurations were established by ECD spectra aided by quantum chemical calculations, which could be beneficial to the structure elucidation of the other similar metabolites. Additionally, some of the isolates exhibited antibacterial activity and brine shrimp toxicity.

Experimental

General experimental procedures

NMR spectra were recorded at 500 and 125 MHz in $CDCl_3$ for ^1H and ^{13}C, respectively, on a Bruker Avance III 500 NMR spectrometer using TMS as the internal standard. The high resolution mass spectra were determined on Autospec Premier P776 and VG Autospec 3000 mass spectrometers. The IR spectra were obtained on a JASCO FT/IR-4100 Fourier transform infrared spectrometer. The UV spectra were measured on a DU 800 Spectrophotometer. The optical rotations were determined on a JASCO *P*-1020 polarimeter. Electronic circular dichroism (ECD) spectra were recorded on a Chirascan CD Spectrometer. Quantum chemical calculations were operated *via* Gaussian 09 software (IA32W-G09RevC.01). HPLC separation was carried out on an Elite HPLC system (P270 pump, UV230+ detector, Dalian Elite Analytical Instruments Co., Ltd, Dalian, China) using an Eclipse XDB-C18 (5 μm, 9.4 × 250 mm) column. Column chromatography was performed with silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Qingdao, China) and Sephadex LH-20 (Pharmacia). TLC was carried out with precoated silica gel plates (GF-254, Qingdao Haiyang Chemical Co., Qingdao, China). All solvents were of analytical grade.

Fungal material and fermentation

The fungal strain *A. ustus* cf-42 was isolated from the fresh tissue of surface-sterilized marine green alga *C. fragile* that was collected from Zhoushan Island in August, 2010. The fungus was identified by morphological observation and analysis of the ITS region of the rDNA, whose sequence data have been deposited at GenBank with the accession number JX036023. The strain was preserved at the Coastal Natural Product Laboratory of the Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences and China Center for Type Culture Collection (No. CCTCC M 2011420).

The initial cultures were maintained on the potato dextrose agar (PDA) plates. Pieces of mycelia were cut into small segments and aseptically inoculated into 50 Erlenmeyer flasks (1 L), each containing 300 mL potato dextrose broth (PDB, 50% sea water) culture media. Static fermentation was performed at room temperature (*ca.* 20 °C) for 35 d.

Extraction and isolation

The whole cultures (300 mL × 50 flasks) were filtered by cheesecloth to separate mycelia from broth. The broth was extracted with EtOAc to give a concentrated extract (5.6 g). The dried mycelia were homogenized and extracted with a mixture of $CHCl_3$ and MeOH (1 : 1, v/v), then the concentrated extract was partitioned between EtOAc and H_2O to yield an EtOAc-soluble extract (29.9 g). These two parts were combined for further separation based on their identical TLC profiles. The total EtOAc-soluble fraction (35.5 g) was subjected to step-gradient silica gel column chromatography (CC) with a solvent system consisting of 0–100% petroleum ether (PE)–EtOAc to afford 14 fractions (Frs. 1–14) on the basis of TLC analysis. Fr. 4 eluted with PE/EtOAc (20 : 1) and was further purified by CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1 : 1) and silica gel (PE/EtOAc, 20 : 1) and preparative HPLC (MeOH/H_2O, 85 : 15) to afford **4** (3.0 mg), **11** (6.0 mg), and a subfraction, which was further purified by preparative TLC (PE/EtOAc, 10 : 1) to yield **7** (2.1 mg). Fr. 5 eluted with PE/EtOAc (10 : 1) and was further purified by CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1 : 1) and silica gel (PE/EtOAc, 10 : 1), preparative HPLC (MeOH/H_2O, 85 : 15), and preparative TLC ($CHCl_3$/EtOAc, 10 : 1) to yield **3** (5.2 mg). Fr. 8 eluted with PE/EtOAc (2 : 1) and was further purified by CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1 : 1) and silica gel (PE/EtOAc, 4 : 1) to give three subfractions (Frs. 8.1–8.3). Fr. 8.1 was further purified by preparative HPLC (MeOH/H_2O, 85 : 15) and preparative TLC ($CHCl_3$/EtOAc, 2 : 1) to yield **17** (5.4 mg). Fr. 8.2 was rechromatographed by CC on silica gel (PE/EtOAc, 5 : 1) to give two subfractions (Frs. 8.2.1 and Fr. 8.2.2). Fr. 8.2.1 was purified by preparative HPLC (MeOH/H_2O, 85 : 15) to yield **18** (9.1 mg). Fr. 8.2.2 was further purified by preparative HPLC (MeOH/H_2O, 85 : 15) and preparative TLC ($CHCl_3$/EtOAc, 3 : 1) to yield **19** (3.1 mg). **13**/**14** (2.3 mg) was

obtained from Fr. 8.2.2 by preparative HPLC (MeOH/H_2O, 85 : 15) and preparative TLC ($CHCl_3$/EtOAc, 4 : 1). Fr. 8.3 was further purified by CC on silica gel (PE/EtOAc, 5 : 1), preparative HPLC (MeOH/H_2O, 85 : 15), and preparative TLC ($CHCl_3$/EtOAc, 3 : 1) to yield **5** (2.8 mg). Fr. 9 was eluted with PE/EtOAc (2 : 1) and was further purified by CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1 : 1) and silica gel (PE/EtOAc, 4 : 1) to give two subfractions (Frs. 9.1 and 9.2). Fr. 9.1 was further purified by preparative HPLC (MeOH/H_2O, 85 : 15) and preparative TLC ($CHCl_3$/EtOAc, 1 : 1) to yield **9** (15.9 mg) and a subfraction, which was further purified by silica gel (PE/EtOAc, 2.5 : 1) to yield **6** (19.7 mg) and **8** (9.0 mg). Fr. 9.2 was rechromatographed by CC on silica gel (PE/EtOAc, 3 : 1) and preparative HPLC (MeOH/H_2O, 85 : 15) to give four subfractions (Frs. 9.2.1–9.2.4). Fr. 9.2.1 was purified by preparative HPLC (MeOH/H_2O, 85 : 15) to yield **10** (39.3 mg). Fr. 9.2.2 was further purified by preparative TLC ($CHCl_3$/EtOAc, 2 : 1) to afford **20** (7.6 mg). Fr. 9.2.3 was purified by preparative HPLC (MeOH/H_2O, 85 : 15), preparative TLC ($CHCl_3$/EtOAc, 2 : 1; PE/EtOAc, 1 : 1) to give **1** (7.0 mg) and **2** (4.4 mg). Fr. 11 eluted with EtOAc and was further purified by CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1 : 1), silica gel (PE/EtOAc, 1 : 1) and preparative TLC ($CHCl_3$/EtOAc,2 : 1,2%HAc) to give **15**/**16** (12.6 mg) and **12** (8.1 mg).

1,2-DIHYDROTERRETONIN F (2). Colorless oil; $[\alpha]_D^{21}$ −31.2 (*c* 0.12, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 275 (3.88) nm; IR (KBr) ν_{max} 3402, 2943, 1739, 1666, 1454, 1369, 1227, 1115, 1045, 756 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HREIMS *m*/*z* 472.2093 $[M]^+$ (calcd for $C_{26}H_{32}O_8$, 472.2097).

(6α)-21-DEOXYOPHIOBOLIN G (3). Colorless oil; $[\alpha]_D^{23}$ +231.6 (*c* 0.22, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 241 (4.45) nm; IR (KBr) ν_{max} 2931, 2866, 1697, 1620, 1446, 1373, 1176, 756 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HREIMS *m*/*z* 352.2769 $[M]^+$ (calcd for $C_{25}H_{36}O$, 352.2766).

(6α)-16,17-DIHYDRO-21-DEOXYOPHIOBOLIN G (4). Colorless oil; $[\alpha]_D^{23}$ +54.1 (*c* 0.17, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (3.67) nm; IR (KBr) ν_{max} 2931, 2870, 1693, 1624, 1446, 1373, 1180, 852 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HREIMS *m*/*z* 354.2925 $[M]^+$ (calcd for $C_{25}H_{38}O$, 354.2923).

OPHIOBOLIN U (5). Colorless oil; $[\alpha]_D^{20}$ +152.3 (*c* 0.18, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 242 (4.25) nm; IR (KBr) ν_{max} 3340, 2931, 2873, 1685, 1620, 1454, 1373, 1011, 756 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRESIMS *m*/*z* 369.2788 $[M + H]^+$ (calcd for $C_{25}H_{37}O_2$, 369.2793).

OPHIOBOLIN V (6). Colorless oil; $[\alpha]_D^{13}$ +170.4 (*c* 0.14, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 246 (4.11) nm; IR (KBr) ν_{max} 3370, 2927, 2866, 1670, 1620, 1446, 1373, 1038, 748 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HREIMS *m*/*z* 368.2722 $[M]^+$ (calcd for $C_{25}H_{36}O_2$, 368.2715).

OPHIOBOLIN W (7). Colorless oil; $[\alpha]_D^{23}$ +0.35 (*c* 0.045, $CHCl_3$); IR (KBr) ν_{max} 3402, 2931, 1454, 1381, 1018, 914 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HREIMS *m*/*z* 374.3179 $[M]^+$ (calcd for $C_{25}H_{42}O_2$, 374.3185).

(6-STROBILACTONE-B) ESTERS OF (E,E)-6,7-EPOXY-2,4-OCTADIENOIC ACIDS (13 AND 14). Colorless oil; UV ($CHCl_3$) λ_{max} (log ε) 266 (4.20) nm; IR (KBr) ν_{max} 3410, 2927, 2870, 1774, 1701, 1635, 1458, 1358, 1234, 1142, 1011, 760 cm^{-1}; ^1H NMR ($CDCl_3$, 125 MHz) δ 1.70 (1H, m, H-1a), 2.12 (1H, m, H-1b), 1.61 (1H, m, H-2a), 1.70 (1H, m, H-2b), 1.30 (1H, m, H-3a), 1.44 (1H, m, H-3b), 2.03 (1H, d, *J* = 4.7 Hz, H-5), 5.74 (1H, brs, H-6), 5.92 (1H, brs, H-7), 4.73 (1H, d, *J* = 12.4 Hz, H-12a), 4.96 (1H, d, *J* = 12.4 Hz, H-12b), 1.00 (1H, s, H-13), 1.13 (1H, s, H-14), 1.19 (1H, s, H-15), 5.89 (1H, d, *J* = 15.8 Hz, H-2′), 7.23 (1H, brdd, *J* = 15.8, 11.1 Hz, H-3′), 6.50 (1H, brdd, *J* = 15.2, 11.1 Hz, H-4′), 5.86/5.87 (1H, dd, *J* = 15.2, 7.5 Hz, H-5′), 3.14 (1H, brd, *J* = 7.5 Hz, H-6′), 2.96 (1H, qd, *J* = 5.1, 2.0 Hz, H-7′), 1.38 (1H, d, *J* = 5.1 Hz, H-8′), 2.47 (1H, brs, OH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 30.3 (CH_2, C-1), 17.8 (CH_2, C-2), 44.8 (CH_2, C-3), 33.9 (C, C-4), 44.8 (CH, C-5), 66.5 (CH, C-6), 123.8 (CH, C-7), 134.9 (C, C-8), 74.7 (C, C-9), 37.9 (C, C-10), 174.8 (C, C-11), 69.0 (CH_2, C-12), 32.5 (CH_3, C-13), 24.8 (CH_3, C-14), 18.5 (CH_3, C-15), 165.8 (C, C-1′), 121.8 (CH, C-2′), 143.8 (CH, C-3′), 130.7/130.8 (CH, C-4′), 139.9 (CH, C-5′), 58.3/58.4 (CH, C-6′), 57.4 (CH, C-7′), 17.6 (CH_3, C-8′); HRESIMS *m*/*z* 425.1945 $[M + Na]^+$ (calcd for $C_{23}H_{30}O_6Na$, 425.1940).

Computational details

The energy-minimized conformers for **2**, **3**, and **12** that matched NOE data were generated by the Dreiding force field in MarvinSketch (optimization limit = normal, diversity limit = 0.1) regardless of rotations of methyl and hydroxyl groups, the geometries of which were further optimized at the B3LYP/6-31G(d) level in methanol. The predominant conformers (Fig. 3) without vibrational imaginary frequencies were subjected to the theoretical calculations of ECD spectra using the time-dependent density function theory (TD-DFT) method at the B3LYP/6-31G(d) level in methanol, which were drawn *via* SpecDic software with sigma = 0.35 and UV shift = −30 nm (magnified by 0.3 times), sigma = 0.25 and UV shift = −10 nm (magnified by 0.1 times), and sigma = 0.25 and UV shift = −10 nm (magnified by 0.2 times) for **2**, **3**, and **12**, respectively.[13] All the above calculations were performed with the integral equation formalism variant polarizable continuum model (IEF-PCM) as implemented in Gaussian 09.

Bioassays

Antibacterial activity against *E. coli* and *S. aureus*, antifungal activity against phytopathogenic *C. lagenarium* and *F. oxysporum*, and toxicity against brine shrimp (*A. salina*) were tested as described previously,[15] with chloramphenicol, amphotericin B, and thyrsiferol as positive controls, respectively.

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