Sesquiterpenoids from the Mangrove-Derived Aspergillus ustus 094102

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Abstract: Four new drimane sesquiterpenoids (1–4) and three known ones (5–7) were isolated from the fermentation broth of the mangrove-derived Aspergillus ustus 094102. Compound 5 was further resolved as four purified compounds 5a–5d. By means of extensive spectroscopic and ECD analysis as well as the chemical transformation, their structures were identified as (2R,3R,5S,9R,10S)-2,3,9,11-tetraydroxydrim-7-en-6-one (ustusol F, 1), (2R,3R,5R,9R,10R)-2,3,11-trihydroxydrim-7-en-6-one (9-deoxyustusol F, 2), (3S,5R,9R,10R)-3,11,12-trihydroxydrim-7-en-6-one (ustusol G, 3), (5S,6R,9S,10S,11R,2′E,4′E)-(11-dideoxy-11-hydroxyestrobilactone A-6-yl)-5-carboxypenta-2,4-dienoate (ustusolate H, 4), ((5S,6R,9S,10S)-strobilactone A-6-yl) (2′E,4′E)-6′,7′-dihydroxyocta-2,4-dienoate (ustusolate I, 5), (2′E,4′E,6′,7′-erythro)-ustusolate I (5a) and (2′E,4′E,9′-ent-6′,7′-erythro)-ustusolate I (5b), (2′E,4′E,6′,9′R,7′R)-ustusolate I (5c) and (2′E,4′E,6′,5′S,7′S)-ustusolate I (5d), (5S,6R,9S,10S,2′E,4′E)-strobilactone A-6-yl)-5-carboxypenta-2,4-dienoate (ustusolate J, 6), and (2S,5S,9R,10S)-2,9,11-trihydroxydrim-7-en-6-one (ustusol B, 7), respectively. Compound 5 showed antiproliferation against the human tumor cells CAL-62 and MG-63 with the IC_{50} values of 16.3 and 10.1 µM, respectively.

Keywords: drimane sesquiterpenoids; absolute configuration; antiproliferation; Aspergillus ustus; mangrove-derived fungus

1. Introduction

As well as we know, microbial metabolites are an important source of drug discovery and development [1]. However, with the deepening of research, many strains in the conventional environment have been repeatedly studied, resulting in the increase of the recurrence rate of known compounds and the decrease of the occurrence rate of new bioactive compounds [2]. Mangrove fungi have attracted much attention because of their special structure and bioactivity of their secondary metabolites, which has become a new hotspot in drug development [3,4]. Our previous work reported 9 drimane sesquiterpenoids, 8 benzofurans [4], and 18 ophiobolins [5] from mangrove-derived fungus Aspergillus ustus 094102, among which ustusorane E and ustusolate E exhibited cytotoxic activity against the HL-60 cells with IC_{50} Values of 0.13 and 9.0 µM, respectively [4]. In addition, more than 50 drimane sesquiterpenoids have been reported from fungi, including cytotoxic
strobilactones A and B from *Strobilurus ohshimae* [6], (6-strobilactone-B) ester of (E,E)-6-oxo-2,4-hexadienoic acid from marine sponge-derived *A. ustus* [7], (6-strobilactone-B) ester of (E,E)-6-carbon-7-hydroxy-2,4-octadienoic acid from mangrove-derived *A. ustus* [8], synergistic antibacterial ustusoic acid B from *A. ustus* [9], and ET-1 binding inhibitory (2′E,4′E,6′E)-6-(l′-carboxycta-2′,4′,6′-triene)-9-hydroxydrim-7-ene-l l-al from *A. ustus* var. *pseudoedeflectus* [10], etc. In order to further explore the new drimane sesquiterpenoids produced by *A. ustus* strain 094102, we continued to study its secondary metabolites. As a result, we isolated and identified four new drimane sesquiterpenoids (1–4), as well as three known analogues, (strobilactone A-6-yl) (2E,4E)-6,7-dihydroxyocta-2,4-dienoate (5) [7] that were further isolated as four isomers 5a–5d, mono(6-strobilactone A) ester of (E,E)-2,4-hexadienedioic acid (6) [7], and 2α,9α,11-trihydroxy-6-oxodrim-7-ene (7) [7]. The structures elucidation including absolute configurations and the antiproliferative activity will be discussed here.

2. Results and Discussion

The bioactive EtOAc extract of the fermentation broth of the mangrove-derived fungus *Aspergillus ustus* 094102 was chromatographed on silica gel, Sephadex LH-20, and preparative HPLC columns to give compounds 1–7 (Figure 1).

![Figure 1. Structures of compounds 1–7 from Aspergillus ustus 094102.](image)

Figure 1. Structures of compounds 1–7 from *Aspergillus ustus* 094102.

Compound 1 was obtained as a colorless oily solid. Its molecular formula was determined as C_{15}H_{24}O_{5} based on the high-resolution mass spectrometry (HRMS, ESI-Orbitrap) peak at m/z 285.1694 [M+H]^{+} or 283.1547 [M–H]^{-} (Figure S1), indicating 4 index of hydrogen deficiency (IHD). The IR spectrum at ν_{max} 3399 and 1663 cm^{-1} (Figure S2), corresponded to a hydroxy and an α,β-unsaturated carbonyl group, respectively. The 1H-NMR data (Table 1, Figure S3) of 1 revealed four tertiary methyl groups at δ_{H} 1.04 (s, H-13/15), 1.14 (s, H-14) and 1.96 (s, H-12), an oxymethylene signal at δ_{H} 3.53/3.64 (d/d, H-11), a methylene signal at δ_{H} 1.85/1.69 (dd/t, H-1), one olefinic proton signal at δ_{H} 5.61 (d, H-7), three methine signals at δ_{H} 3.47 (dt, H-2), 2.67 (d, H-3) and 2.81 (s, H-5), as well as four exchangeable proton signals at δ_{H} 4.48 (HO-3/2), 4.91 (HO-11) and 5.06 (HO-9). The 13C-NMR and DEPT data (Table 1, Figures S4 and S5) of 1 revealed 15 carbon signals, including a ketone carbonyl signal at δ_{C} 199.2 (C-6), two olefinic carbons at δ_{C} 128.2/157.4 (C-7/C-8), four methyl signals at δ_{C} 16.7/19.1/19.2/29.3 (C-15/C-13/C-12/C-14), two methylene at δ_{C} 38.6/61.9 (C-1/C-11), three methines at δ_{C} 55.0/66.4/81.6 (C-5/C-2/C-3) and three nonhydrogenated carbons at δ_{C} 37.8/45.4/74.6 (C-4/C-10/C-9). These NMR data (Table 1) were closely related to those of 3β,9α,11-trihydroxydrim-7-en-6-one (that is 3β,9α,11-trihydroxy-6-oxodrim-7-ene [7]), indicating the presence of a drimane sesquiterpene skeleton. The key difference was that compound 1 possessed an additional hydroxy group that resided at C-2 of ring A. On the basis of correlations in the COSY experiments between HO-3/H-3, H-3/H-2/H-1 and HO-11/H-11, as well
as the key HMBC correlations from H-1 to C-5/C-10/C-13, H-3 to C-4/C-14/C-15, H-5 to C-4/C-6/C-9/C-10/C-13/C-14/C-15, H-7 to C-5/C-9/C-12, H-11 to C-8/C-9/C-10, H-12 to C-7/C-8/C-9, H-13 to C-5/C-9/C-10, H-14 to C-3/C-4/C-5/C-15, and H-15 to C-3/C-4/C-14 (Figures 2 and S6–S8) further confirmed the constitution of 1 (Figure 1).

The relative configuration was deduced from the NOESY spectrum (Figures 3 and S9), which showed correlations of H-1α to H-3/H-5/HO-9, H-11 to H-1β/H-2/H-13, and H-2 to H-13 indicated cis-orientation of HO-2/H-5/HO-9, and H-2/HO-3/CH3-13/CH2-11, and a trans-fused decalin nucleus. The absolute configuration of 1 was determined by its ECD spectrum. On the basis of the octant rule for cyclohexenones [11–13], the positive Cotton effect at λmax 336 nm (Δε + 8.4) and the negative Cotton effect at λmax 240 nm (Δε − 41.3) (Figures 3 and S10) indicated the (2R,3R,5S,9R,10S)-configuration, consistent with the core configuration of the drimane sesquiterpene, 9α,11-dihydroxydrim-7-en-6-one (that is 6-oxo-7-drimen-9α,11-diol [14]), whose absolute configurations have been established by chemical synthesis. Therefore, compound 1, which we named ustusol F, was determined as (2R,3R,5S,9R,10S)-2,3,9,11-tetrahydroxydrim-7-en-6-one.

Table 1. 1H (500 MHz) and 13C (125 MHz) NMR Data for Compounds 1–3 and 7 (DMSO-d6, TMS, δ ppm).

| Position | δC, type | δH, Mult. (J in Hz) | δC, Type | δH, Mult. (J in Hz) | δC, Type | δH, Mult. (J in Hz) |
|----------|----------|---------------------|----------|---------------------|----------|---------------------|
| 1        | 38.6, CH2 | β 1.69, dd (12.6, 4.6) | 45.3, CH2 | 36.6, CH2 | 41.0, CH2 | 1.71–1.65, m |
|          |          | α 1.85, dd (12.6, 12.1) |          |          |          | 1.76–1.71, m |
| 2        | 66.4, CH | 3.46–3.48, m | 66.1, CH | 26.7, CH2 | 62.4, CH | 3.68–3.72, m |
|          |          |          |          |          |          | 0.96, t (11.9) |
|          |          |          |          |          |          | 1.50, dd (11.9, 3.8) |
| 3        | 81.6, CH | 2.67, d (9.5) | 81.6, CH | 76.8, CH | 51.7, CH2 | 1.50, dd (11.9, 3.8) |
| 4        | 37.8, C | 2.22, s | 38.0, C | 37.5, C | 33.4, C | 2.15, s |
| 5        | 55.0, CH | 2.22, s | 61.6, CH | 62.0, CH | 54.7, CH | 33.4, C |
| 6        | 59.9, CH | 5.71, s | 198.6, C | 199.4, C | 199.6, C | 5.96, s |
| 7        | 128.2, CH | 2.29, br s | 127.9, CH | 123.7, CH | 128.1, CH | 5.61, s |
| 8        | 157.5, C | 42.3, C | 159.0, C | 162.3, C | 157.6, C | 4.26, d (18.1) |
| 9        | 74.6, C | 0.96, s | 57.1, CH | 55.1, CH | 74.6, C | 1.04, s |
| 10       | 45.4, C | 1.04, s | 42.3, C | 41.7, C | 46.2, C | 1.04, s |
| 11       | 61.9, CH2 | 3.53, d (11.5) | 57.9, CH2 | 57.7, CH2 | 61.9, CH2 | 3.53, d (11.5) |
|          |          | 3.64, d (11.5) |          |          |          | 3.64, d (11.5) |
|          |          | 3.74, br d (11.4) |          |          |          | 3.74, br d (11.4) |
| 12       | 19.2, CH3 | 1.98, s | 21.5, CH3 | 61.3, CH2 | 19.2, CH3 | 1.98, s |
| 13       | 16.7, CH3 | 0.88, s | 16.7, CH3 | 15.8, CH3 | 18.9, CH3 | 1.08, s |
| 14       | 29.3, CH3 | 1.12, s | 28.7, CH3 | 28.5, CH3 | 33.8, CH3 | 1.14, s |
| 15       | 19.1, CH3 | 1.03, s | 16.5, CH3 | 15.5, CH3 | 22.7, CH3 | 1.03, s |
| 2-OH     | 4.47, s | 4.47, s | 4.47, s | 10.9 | 4.39, s | 5.02, s |
| 3-OH     | 4.50, s | 4.50, s | 4.50, s |          |          |          |
| 9-OH     | 5.06, s | 5.06, s | 5.06, s |          |          |          |
| 11-OH    | 4.91, s | 4.91, s | 4.91, s |          |          |          |
Compound 2 was obtained as a light-yellow oil. Its molecular formula was determined as C_{15}H_{24}O_{4} based on the HRESIMS peak at m/z 269.1751 [M+H]^+ (Figure S11). The similar IR and UV absorptions to those of 1 implied that they shared the same molecular skeleton (Figure S12). The 1D NMR data (Table 1, Figures S13–S15) were also similar to 1 except for a methine signal at δ_{C/H} 57.1/2.29 which replaced the nonhydrogenated oxycarbon at δ_{C} 74.6, the disappearance of a hydroxy signal at δ_{H} 5.06 (HO-9), and the changes of chemical shifts around C-9. These data combined with the 16 amu less of molecular weight than 1 revealed compound 2 as the 9-deoxy derivative of compound 1. Key COSY of H-9/H-11/HO-11 and HMBC of H-11 to C-8 and C-10 and H-9 to C-10 (Figures 2, S16 and S18) supported the inference. The same relative configuration to 1 was deduced from the NOESY correlations of H-2 (δ_{H} 3.45) to H-13 (δ_{H} 0.88), H-15 (δ_{H} 1.03) and H-1_β (δ_{H} 2.09), H-1α (δ_{H} 1.33) to H-3 (δ_{H} 2.75), H-5 (δ_{H} 2.22) and H-9 (δ_{H} 2.29), and H-3 to H-14 (δ_{H} 1.12) (Figures 3, S16 and S18). The absolute configuration of the threo-2,3-diol in 2 was assigned.
by a dimolybdenum-induced ECD method [15,16]. Upon addition of Mo$_2$(OAc)$_4$ to a DMSO solution of compound 2, a chiral dimolybdenum complex was generated in situ as an auxiliary chromophore. Because the contribution from the inherent ECD was subtracted to give the induced ECD of the complex, the observed sign of the Cotton effect in the induced spectrum originates solely from the chirality of the ortho-diol moiety expressed by the sign of the O-C-C-O torsion angle. The positive Cotton effect at $\lambda_{\text{max}}$ 332 ($\Delta \epsilon + 6.8$) nm (Figure S20) permitted us to assign the (2R,3R)-configuration on the basis of Snatzke’s empirical rule [15]. In addition, compounds 1 and 2 also showed a similar ECD Cotton effect, indicating the same absolute configuration. Thus, compound 2, which we named 9-deoxyustusol F, was determined as (2R,3R,5R,9R,10R)-2,3,11-trihydroxydrim-7-en-6-one.

Compound 3 was obtained as a colorless oily solid. Its molecular formula was determined as C$_{15}$H$_{24}$O$_7$ based on the HRESIMS peak at $m/z$ 269.1750 [M+H]$^+$ (Figure S21), indicating an isomer of 2. Similar 1D NMR data (Table 1, Figures S23–S25) with 2 were observed. In addition, a methylene signal ($\delta_{H/C}$ 1.47/26.7) and an oxymethylene signal ($\delta_{H/C}$ 4.19/4.26/61.3) replaced the methyl signal ($\delta_{H/C}$ 1.98/21.5) and oxymethine signal ($\delta_{H/C}$ 3.45/66.1). Key COSY of H-1/H$_2$-2/H-3 as well as the HMBC of H$_2$-2 ($\delta_H$ 4.19/4.26) to C-8 ($\delta_C$ 162.3), H-7 ($\delta_H$ 5.96) to C-12 ($\delta_C$ 61.3) and H$_2$-2 ($\delta_H$ 1.47) to C-4 ($\delta_C$ 37.5) and C-10 ($\delta_C$ 41.7) revealed that 2-CH$_2$ was moved to C-12 to form 2-CH$_2$-OH, respectively (Figures 2, S26 and S28). The relative configuration of compound 3 was deduced from the NOE difference (NOEdiff) experiment. NOEdiff of 3 showed that H-5 ($\delta_H$ 2.15) and H-1a ($\delta_H$ 1.44) were enhanced after the irradiation of H-9 ($\delta_H$ 2.31), while H-3 ($\delta_H$ 3.02) and H-9 ($\delta_H$ 2.31) were enhanced after the irradiation of H-5. The NOE enhancements of H-3 ($\delta_H$ 3.02) and H-5 ($\delta_H$ 2.15) were also observed after H-14 ($\delta_H$ 1.10) was irradiated, while H-13 ($\delta_H$ 0.80) was enhanced after the irradiation of H-15 ($\delta_H$ 0.99). H-1b ($\delta_H$ 1.88) and H-15 was enhanced after the irradiation of H-13 (Figure S29). These NOE data indicated the cis-orientation of H-3, H-5, H-9 and H-14 as well as H-13 and H-15, indicating the same relative configuration of 3 to 2 in the chiral centers of C-3, C-5, C-9, and C-10. The similar ECD spectrum to that of 2 implied the same absolute configuration, which was confirmed by octant rule for cyclohexanone [11–13], the positive Cotton effect at $\lambda_{\text{max}}$ 335 nm ($\Delta \epsilon + 10.6$) and the negative Cotton effect at $\lambda_{\text{max}}$ 241 nm ($\Delta \epsilon - 19.1$) (Figures 4 and S30). Accordingly, compound 3, which we named ustusolate G, was elucidated as (3S,5R,9R,10R)-3,11,12-trihydroxydrim-7-en-6-one.

![Figure 4. The preparation of acetonide 5e from 5a.](image)

Compound 4 was obtained as a colorless solid. Its molecular formula was determined as C$_{21}$H$_{28}$O$_7$ based on the HRESIMS peak at $m/z$ 391.1762 [M–H]$^-$, indicating 8 HIDs (Figure S32). The IR spectrum showed absorption bands of hydroxyl and conjugated carbonyl at $\nu_{\text{max}}$ 3434 and 1696 cm$^{-1}$ (Figure S33), respectively. The 1D NMR spectra of 4 (Table 2, Figures S34–S36) were very similar to those of (2E,4E)-(strobilactone A-6-yl)-5-carboxypenta-2,4-dienoate (that is mono(6-strobilactone B) ester of (E,E)-2,4-hexadienedioic acid [7]), which we named ustusolate J (6) for convenience, suggesting that they shared the same molecular scaffold. The only difference was a replacement of the lactone carbonyl signal ($\delta_C$ 174.6 in 6) by the hemiacetal methine group ($\delta_C$ 197.4/5.20 in 4). In addition, the chemical shifts for C-9 and C-7 have a great increase and decrease, respectively (Table 2 and Figure S35). These data combined with a 2 amu more than 6 suggested that the $\gamma$-
lactone of 6 was reduced to the corresponding hemiacetal in 4. The key HMBC correlations from hemiacetal proton (δH-C11 5.20) to C-9 (δC 66.4)/C-10 (δC 38.0)/C-12 (δC 65.8), from H-12 (δH 4.08/4.38) to C-7 (δC 117.0)/C-8 (δC 143.2)/C-9/C-11 (δC 97.3), and from H-7 (δH 5.49) to C-5 (δC 45.1)/C-9 verified the deduction (Figures 2 and S39). Compound 4 displayed the key NOESY correlations of H-6 (δH 5.58) with H-5 (δH 2.07) and H-14 (δH 0.91), H-5 with H-1b (δH 1.86) and H-2a (δH 1.42), H-11 (δH 5.20) with H-1a (δH 1.22), as well as H-13 (δH 1.12) with H-2b (δH 1.58) (Figures 3 and S40), indicating cis-orientation of H-5 with H-6 and trans-orientation of H-5 with H-11 and H-13 which is the same relative configuration of 4 to 1 and 6 in the decalin (decahydronaphthalene) nucleus. The same relative configuration of HO-9 was deduced from the same biosynthetic pathway to those of compounds 1 and 5–7. Subsequently, the same ECD pattern of 4–6 (Figure S78) implied the same absolute configuration of the drimane nucleus. Compound 4, which we named ustusolate H, was thus elucidated as (5S,6R,9S,10S,11R,2′E,4′E)-(11-deoxy-11-hydroxystrobi lactone A-6-yl)-5-carboxypenta-2,4-dienoate.

**Table 2.** $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR Data for Compounds 4 and 6 (DMSO-$d_6$, TMS, δ ppm).

| Position | δC, Type | δH, Mult. (J in Hz) | δC, Type | δH, Mult. (J in Hz) |
|----------|----------|---------------------|----------|---------------------|
| 4        |          |                     | 6        |                     |
| 1        | 31.6, CH$_2$ | 1.20–1.23, m | 29.8, CH$_2$ | 1.82, br d (13.5) |
|          | 1.86, td (13.5, 4.1) | 1.95, td (13.5, 4.1) |                     |                     |
| 2        | 17.8, CH$_2$ | 1.39–1.45, m | 17.6, CH$_2$ | 1.43–1.50, m |
|          | 1.52–1.63, m | 1.54–1.64, m |                     |                     |
| 3        | 44.5, CH$_2$ | 1.17–1.20, m | 44.4, CH$_2$ | 1.19, d (12.5) |
|          | 1.29–1.35, m | 1.34, br d (12.5) |                     |                     |
| 4        | 33.3, C | 33.5, C |                     |                     |
| 5        | 45.1, CH | 2.07, d (4.6) | 44.6, CH | 2.01, d (4.7) |
|          |                     |                     | 68.4, CH | 5.79, br s |
| 6        | 117.0, CH | 5.49, d (2.3) | 121.3, CH | 5.60, br s |
| 7        | 143.2, C | 142.3, C |                     |                     |
| 8        | 76.4, C | 73.3, C |                     |                     |
| 9        | 38.0, C | 37.4, C |                     |                     |
| 10       | 97.4, CH | 5.20, s | 174.6, C |                     |
| 11       | 65.8, CH$_2$ | 4.08, d (13.0) | 66.6, CH$_2$ | 4.78, d (12.7) |
|          | 4.38, d (13.0) |                     | 4.87, d (12.7) |                     |
| 12       | 18.6, CH$_3$ | 1.12, s | 18.5, CH$_3$ | 1.05, s |
|          | 32.7, CH$_3$ | 0.91, s | 24.5, CH$_3$ | 1.05, s |
| 14       | 24.5, CH$_3$ | 1.06, s | 32.3, CH$_3$ | 0.91, s |
| 15       | 165.0, C | 165.0, C |                     |                     |
| 2'       | 128.2, CH | 6.39, dd (11.6, 2.9) | 127.9, CH | 6.33–6.43, overlap $^a$ |
|          | 140.6, CH | 7.32, dd (11.2, 2.9) | 137.0, CH | 7.27–7.35, overlap $^b$ |
| 3'       | 141.9, CH | 7.29, dd (11.2, 2.9) | 140.6, CH | 7.27–7.35, overlap $^b$ |
| 4'       | 130.2, CH | 6.35, dd (11.6, 2.9) | 130.4, CH | 6.33–6.43, overlap $^a$ |
| 5'       | 166.9, C | 166.9, C |                     |                     |

$^a$ Overlapping signals of H-2' with H-5'; $^b$ Overlapping signals of H-3' with H-4'.

Compound 5 was obtained as a yellow oil. Its molecular formula was determined as C$_{21}$H$_{32}$O$_7$ based on the ESIMS peak at m/z 419.1 for [M–H]$^-$ and m/z 464.9 for [M + HCO$_2$]$^+$ (Figure S42), indicating 8 HIDs. A literature search verified that the constitution (planar structure) of compound 5 was the same as the (strobi lactone A-6-yl) (2E,4E)-6,7-dihydroxyocta-2,4-dienoate (that is 6-strobi lactone-B) esters of (E,E)-6,7-dihydroxy-2,4-octadienoic acid [7], for almost the same NMR data. However, four sets of $^{13}$C NMR signals of compound 5 (Figure S40) for the side chain at δC 165.51/165.50/165.49/165.47 (C-1'), 120.03/120.99/119.95/119.90 (C-2'), 145.41/145.37/145.43/145.26 (C-3'), 127.54/127.35/127.16/126.98 (C-4'), 146.18/146.12/145.48/145.45 (C-5'), 75.16/75.00/74.64/74.46 (C-6'), 69.64/69.62/69.33/69.32 (C-7'), and 19.34/19.26/18.26/18.24 (C-8') were observed, indi-
cating four stereoisomers of 5 resulted from the ortho-diol chiral centers of the side chain. With the help of HPLC, compound 5, which we named ustusolate I for convenience, was confirmed to have four baseline-separated peaks, then purified 5a, 5b, 5c, and 5d were obtained (Figure S83). The NMR differences of 5a–5d were concentrated in the side chains (Tables 3 and 4, Figures S45–S60), and indicated that compounds 5a and 5b, 5c, and 5d were two pairs of enantiomers of the ortho-diol in the side chain. To elucidate the relative configuration of 6′,7′-diol moiety, the acetone (5e) was prepared from 5a (Figure 4). The 1D and 2D NMR spectra (Tables 3 and 4, Figures S66–S70), as well as the NOESY correlations of H-5′ (δ_H_6.24)/H-8′ (δ_H_1.01) and H-3′-11′ (δ_H_4.62)/H-7′ (δ_H 4.34) and H-5′/H-10′ (δ_H 1.28) in 5e (Figures 3 and S71) clearly suggested an erythro-6′,7′-diol in 5a and 5b, and a threo-6′,7′-diol in 5c and 5d was accordingly elucidated. This conclusion is consistent with the chemical shift rule of methyl carbon (δCH3) for 1-methyl-1,2-diol by chemical synthesis, that is 18.1–18.6 and 19.1–19.6 ppm for threo- and erythro-1,2-diol, respectively [17]. The absolute configuration of the threo-6′,7′-diol in 5c and 5d was assigned by a dimolybdenum-induced ECD method [15,16] in the same manner as that of compound 2. Upon addition of Mo2(OAc)4 to a solution of compounds 5c and 5d in DMSO, a chiral dimolybdenum complex was generated in situ as an auxiliary chromophore. According to the negative ECD Cotton effects of 5e at 1303 (Δε = 7.9) nm and the positive ECD Cotton effects of compound 5d at 1303 (Δε = 2.37) nm (Figures S73 and S74), the absolute configuration of threo-6′,7′-diol in 5c and 5d were determined to be (6′R,7′R) and (6′S,7′S), respectively. Thus, the structure of 5c and 5d was unambiguously determined as (2′E,4′E,6′,7′R)-ustusolate I (5c) and (2′E,4′E,6′,7′S)-ustusolate I (5d), respectively. Unfortunately, the absolute configuration of compounds 5a and 5b were not determined yet in this paper, which we tentatively named (2′E,4′E,6′,7′-erythro)-ustusolate I (5a) and (2′E,4′E,ent-6′,7′-erythro)-ustusolate I (5b), respectively.

Table 3. 1H NMR Data for Compounds 5a–5e (600 MHz, DMSO-d6, TMS, δ ppm).

| Position | 5a | 5b | 5c | 5d | 5e |
|----------|----|----|----|----|----|
| δ_H, Mult. (J in Hz) | δ_H, Mult. (J in Hz) | δ_H, Mult. (J in Hz) | δ_H, Mult. (J in Hz) | δ_H, Mult. (J in Hz) |
| 1a | 1.83, d (13.6) | 1.83, d (13.6) | 1.84, d (13.6) | 1.84, d (13.6) | 1.83, d (13.7) |
| 1b | 1.95, dd (13.7, 4.3) | 1.96, dd (13.7, 4.3) | 1.96, dd (13.8, 4.2) | 1.96, dd (13.7, 4.4) | 1.96, dd (13.8, 4.4) |
| 2a | 1.48, dt (13.7, 3.9) | 1.48, dt (13.7, 3.8) | 1.48, dt (13.7, 3.8) | 1.47, dt (13.7, 3.8) | 1.45–1.49, m |
| 2b | 1.56–1.66, m | 1.56–1.66, m | 1.57–1.65, m | 1.57–1.65, m | 1.56–1.62, m |
| 3a | 1.21, td (13.3, 3.4) | 1.21, td (13.3, 3.4) | 1.20, td (13.3, 3.5) | 1.21, td (13.3, 3.4) | 1.18–1.23, m |
| 3b | 1.34, d (12.7) | 1.34, d (12.7) | 1.34, d (12.7) | 1.34, d (12.7) | 1.34, d (12.5) |
| 5 | 2.02, d (4.9) | 2.01, d (5.0) | 2.01, d (5.0) | 2.01, d (4.9) | 2.01, d (5.0) |
| 6 | 5.59, br s | 5.59, br s | 5.59, br s | 5.59, br s | 5.59, br s |
| 7 | 5.79, br s | 5.79, br s | 5.79, br s | 5.79, br s | 5.79, br s |
| 12a | 4.79, d (12.6) | 4.79, d (12.7) | 4.79, d (12.6) | 4.79, d (12.6) | 4.78, d (12.7) |
| 12b | 4.88, dt (12.6, 2.4) | 4.88, dt (12.6, 2.4) | 4.88, dt (12.6, 2.4) | 4.88, dt (12.6, 2.4) | 4.88, dt (12.6, 2.5) |
| 13 | 1.06, s | 1.06, s | 1.06, s | 1.06, s | 1.06, s |
| 14 | 0.92, s | 0.92, s | 0.92, s | 0.92, s | 0.92, s |
| 15 | 1.07, s | 1.07, s | 1.07, s | 1.07, s | 1.07, s |
| 2′ | 5.94, d (15.3) | 5.94, d (15.3) | 5.94, d (15.3) | 5.94, d (15.3) | 6.01, d (15.3) |
| 3′ | 7.22, dd (15.3, 10.7) | 7.22, dd (15.4, 10.7) | 7.23, dd (15.3, 11.1) | 7.23, dd (15.3, 11.1) | 7.27, dd (15.3, 11.0) |
| 4′ | 6.43, dd (15.3, 10.7) | 6.42, dd (15.3, 10.8) | 6.46, dd (15.3, 11.1) | 6.45, dd (15.3, 11.2) | 6.47, dd (15.2, 11.1) |
| 5′ | 6.36, dd (15.3, 4.9) | 6.34, dd (15.3, 5.1) | 6.32, dd (15.3, 4.9) | 6.30, dd (15.3, 5.1) | 6.23, dd (15.2, 6.6) |
| 6′ | 3.86, dd (10.2, 5.0) | 3.84, dd (10.2, 5.1) | 3.98, dd (10.2, 5.0) | 3.96, dd (10.2, 5.1) | 4.62, dd (12.8, 6.5) |
| 7′ | 3.48, dq (11.6, 6.3) | 3.48, dq (11.6, 6.3) | 3.57, dq (11.6, 6.3) | 3.57, dq (11.6, 6.3) | 4.34, dq (12.8, 6.4) |
| 8′ | 1.03, d (6.3) | 1.03, d (6.3) | 0.95, d (6.3) | 0.95, d (6.3) | 1.01, d (6.4) |
| 9-OH | 6.29, s | 6.28, s | 6.29, s | 6.29, s | 6.30, s |
| 6′-OH | 4.99, d (5.3) | 5.00, d (5.2) | 5.01, d (4.7) | 5.02, d (4.7) | 1.28, s |
| 7′-OH | 4.60, d (5.3) | 4.60, d (5.3) | 4.66, d (4.7) | 4.65, d (4.7) | 1.40, s |
Table 4. $^{13}$C NMR Data for Compounds 5a–5e (150 MHz, DMSO-$d_6$, TMS, $\delta$ ppm).

| Position | 5a $\delta_C$, Type | 5b $\delta_C$, Type | 5c $\delta_C$, Type | 5d $\delta_C$, Type | 5e $\delta_C$, Type |
|----------|---------------------|---------------------|---------------------|---------------------|---------------------|
| 1        | 29.6, CH$_2$        | 29.6, CH$_2$        | 29.6, CH$_2$        | 29.6, CH$_2$        | 29.6, CH$_2$        |
| 2        | 17.5, CH$_2$        | 17.5, CH$_2$        | 17.5, CH$_2$        | 17.5, CH$_2$        | 17.5, CH$_2$        |
| 3        | 44.5, CH$_2$        | 44.5, CH$_2$        | 44.5, CH$_2$        | 44.5, CH$_2$        | 44.5, CH$_2$        |
| 4        | 33.3, C             | 33.3, C             | 33.4, C             | 33.4, C             | 33.4, C             |
| 5        | 44.2, CH$_2$        | 44.2, CH$_2$        | 44.2, CH$_2$        | 44.2, CH$_2$        | 44.2, CH$_2$        |
| 6        | 65.8, CH$_2$        | 65.8, CH$_2$        | 65.8, CH$_2$        | 65.8, CH$_2$        | 65.8, CH$_2$        |
| 7        | 121.4, CH$_2$       | 121.4, CH$_2$       | 121.4, CH$_2$       | 121.4, CH$_2$       | 121.4, CH$_2$       |
| 8        | 137.2, C            | 136.6, C            | 136.6, C            | 136.6, C            | 136.7, C            |
| 9        | 73.2, C             | 73.2, C             | 73.2, C             | 73.2, C             | 73.2, C             |
| 10       | 37.3, C             | 37.3, C             | 37.3, C             | 37.3, C             | 37.3, C             |
| 11       | 174.4, C            | 174.4, C            | 174.4, C            | 174.4, C            | 174.4, C            |
| 12       | 68.3, CH$_2$        | 68.2, CH$_2$        | 68.3, CH$_2$        | 68.3, CH$_2$        | 68.3, CH$_2$        |
| 13       | 18.3, CH$_3$        | 18.3, CH$_3$        | 18.3, CH$_3$        | 18.3, CH$_3$        | 18.3, CH$_3$        |
| 14       | 32.2, CH$_3$        | 32.2, CH$_3$        | 32.2, CH$_3$        | 32.2, CH$_3$        | 32.2, CH$_3$        |
| 15       | 24.3, CH$_3$        | 24.3, CH$_3$        | 24.3, CH$_3$        | 24.3, CH$_3$        | 24.3, CH$_3$        |
| 1'       | 165.5, C            | 165.5, C            | 165.5, C            | 165.5, C            | 165.5, C            |
| 2'       | 119.9, CH$_2$       | 120.0, CH$_2$       | 119.9, CH$_2$       | 120.0, CH$_2$       | 121.4, CH$_2$       |
| 3'       | 145.5, CH$_2$       | 145.4, CH$_2$       | 145.3, CH$_2$       | 145.3, CH$_2$       | 144.6, CH$_2$       |
| 4'       | 126.9, CH$_2$       | 127.1, CH$_2$       | 127.3, CH$_2$       | 127.5, CH$_2$       | 129.2, CH$_2$       |
| 5'       | 146.2, CH$_2$       | 146.1, CH$_2$       | 145.4, CH$_2$       | 145.4, CH$_2$       | 140.4, CH$_2$       |
| 6'       | 75.0, CH$_2$        | 75.1, CH$_2$        | 74.4, CH$_2$        | 74.6, CH$_2$        | 77.7, CH$_2$        |
| 7'       | 69.6, CH$_2$        | 69.6, CH$_2$        | 69.3, CH$_2$        | 69.3, CH$_2$        | 73.5, CH$_2$        |
| 8'       | 19.2, CH$_3$        | 19.3, CH$_3$        | 18.3, CH$_3$        | 18.3, CH$_3$        | 16.0, CH$_3$        |
| 9'       |                     |                     |                     |                     | 107.6, CH$_3$       |
| 10'      |                     |                     |                     |                     | 25.4, CH$_3$        |
| 11'      |                     |                     |                     |                     | 28.0, CH$_3$        |

Compounds 6 and 7 which could be a 3-deoxy derivative of ustusol F (1) were identified by respective comparison of NMR data with those of mono(6-strobilactone-B) ester of (E,E)-2,4-hexadienedioic acid [7] and 2α,9α,11-trihydroxy-6-oxodrim-7-ene [7]. The same ECD pattern of compound 6 with 5 (Figure S78) and compound 7 with 1 (Figure S31) indicated they shared the same absolute configuration. Thus, compounds 6 and 7 were respectively identified as (5S,6R,9S,10S,2’E,4’E)-(strobilactone A-6-yl)-5-carboxypenta-2,4-dienoate (ustusolate J, 6) and (2S,5S,9R,10S)-2,9,11-trihydroxydrim-7-ene-6-one (ustusol B, 7) in this paper. In addition, compound 7 showed almost the same NMR data as our previously reported ustusol B [4] (Table S1) and displayed the same retention times in the co-HPLC (Figure S91). Thus, the structure of ustusol B was revised as structure 7, which was named ustusol B.

The drimane sesquiterpenoids 1–7 were postulated to be biosynthesized from farnesyl-PP (I) which generated intermediate II, III and IV after cyclization and oxidation. The intermediates II and III were subjected to further oxidation to form compounds 1, 2, 3, and 7. The intermediate II was further oxidized to intermediate IV, and the latter was subjected to oxidation, hemi acetalization, and esterification to form compounds 4, 5, and 6 (Figure 5).

The antiproliferations of compounds 1–7 were evaluated against 29 human cancer cell lines and a normal cell line (the names of cell lines are listed in the Supplementary Files) by the cell counting Kit-8 (CCK-8) methods [18,19]. Only compound 5, the mixture of 5a / 5b / 5c / 5d, showed antiproliferative activity against the human thyroid cancer cells (CAL-62) and human osteosarcoma cells (MG-63) with the IC$_{50}$ values of 16.28 ± 1.01 and 10.08 ± 0.04 µM, respectively, while the pure compounds 5a–5d were inactive (IC$_{50}$ ≥ 50 µM). The IC$_{50}$ values of doxorubicin (positive control) against CAL-62 and MG-63 were 0.062 ± 0.022 and 0.096 ± 0.012 µM, respectively. The bacteriostatic activities of compounds 1–7 against 6 human pathogenic bacteria and 6 aquatic pathogenic bacteria (the names are listed in the Supplementary Files) were tested by the diffusion method of
filter paper, but no inhibition zone was observed at the concentration of 100 µg/mL for compounds 1–7.

4′ 126.9, CH 127.1, CH 127.3, CH 127.5, CH 129.2, CH 5′ 146.2, CH 146.1, CH 145.4, CH 145.4, CH 140.4, CH 6′ 75.0, CH 75.1, CH 74.4, CH 74.6, CH 77.7, CH 7 69.6, CH 69.6, CH 69.3, CH 69.3, CH 73.5, CH 8′ 19.2, CH 3 19.3, CH 3 18.3, CH 3 18.3, CH 3 16.0, CH 3 9′ 107.6, CH 3 10 25.4, CH 3 11 28.0, CH 3 12

Compounds 6 and 7 which could be a 3-deoxy derivative of ustusol F (1) were identified by respective comparison of NMR data with those of mono(6-strobilactone-B) ester of (E, E)-2,4-hexadienedioic acid [7] and 2α,9α,11-trihydroxydrim-7-ene [7]. The same ECD pattern of compound 6 with 5 (Figure S78) and compound 7 with 1 (Figure S31) indicated they shared the same absolute configuration. Thus, compounds 6 and 7 were respectively identified as (5S,6R,9S,10S,2′E,4′E)-(strobilactone A-6-yl)-5-carboxypenta-2,4-dienoate (ustusolate J, 6) and (2S,5S,9R,10S)-2,9,11-trihydroxydrim-7-en-6-one (ustusol B, 7) in this paper. In addition, compound 7 showed almost the same NMR data as our previously reported ustusol B [4] (Table S1) and displayed the same retention times in the co-HPLC (Figure S91). Thus, the structure of ustusol B was revised as structure 7, which was named ustusol B.

The drimane sesquiterpenoids 1–7 were postulated to be biosynthesized from farnesyl-PP (I) which generated intermediate II, III, and IV after cyclization and oxidation. The intermediates II and III were subjected to further oxidation to form compounds 1, 2, 3, and 7. The intermediate II was further oxidized to intermediate IV, and the latter was subjected to oxidation, hemi acetalization, and esterification to form compounds 4, 5, and 6 (Figure 5).

Figure 5. Proposed biosynthetic pathway for drimane sesquiterpenoids from A. ustus 094102.

3. Experimental Section
3.1. General Experimental Procedures

Optical rotations were measured with a JASCO P-1020 digital polarimeter. UV data were recorded with a Beckman DU 640 spectrophotometer, and ECD data were collected using a JASCO J-715 spectropolarimeter. IR spectra were taken on a Nicolet NEXUS 470 spectrophotometer as KBr disks. 1H, 13C, DEPT, HMQC, HMBC, COSY, and NOESY NMR spectra were recorded on a JEOL JNM-ECP 600 spectrometer or a Bruker Avance 500 spectrometer in DMSO-d6 solution and were referenced to the corresponding residual solvent signals (δH/C 2.50/39.52 for DMSO-d6). HRESIMS spectra were collected using a Q-TOF Ultima Global GAA076 LC mass spectrometer. ESIMS data were measured using a Waters ACQUITY SQD 2 UPLC/MS system with a reversed-phase C18 column (ACQUITY UPLC BEH C18, 2.1 × 50 mm, 1.7 µm) at a flow rate of 0.4 mL/min. Semipreparative HPLC was performed using an ODS column (YMC-pack ODS-A, 10 × 250 mm, 5 µm, 4 mL/min) and a phenyl column (YMC-pack Ph, 10 × 250 mm, 5 µm, 4 mL/min). Vacuum–liquid chromatography (VLC) utilized silica gel H (Qingdao Marine Chemical Factory, Qingdao, China). TLC were carried out by plates precoated with silica gel GF254 (10–40 µm, Qingdao Marine Chemical Factory) and Sephadex LH-20 (Amersham Pharmacia Biotech, Buckinghamshire, UK) were used for column chromatography (CC).
3.2. Fungal Material

The mangrove fungal strain *A. ustus* 094102 was isolated from the rhizosphere soil of the mangrove plant *Bruguiera gymnorrhiza* grown in Wenchang, Hainan Province of China. It was identified according to the morphological characteristics and the ITS sequences [4,5].

3.3. Cultivation and Extraction

The fungus *A. ustus* 094102 was statically cultured at 25 °C for 28 days in one hundred 1000 mL conical flasks, each containing 300 mL of the liquid medium that was prepared by dissolving maltose (20 g), mannitol (20 g), glucose (10 g), monosodium glutamate (10 g), yeast extract (3 g), corn steep liquor (1 g), CaCO$_3$ (2 g), KH$_2$PO$_4$ (0.5 g), MgSO$_4$·7H$_2$O (0.3 g), and sea salt (33 g) in 1 L of tap water (pH 7.0). The whole fermentation broth (30 L) was filtered by cheesecloth to separate the mycelia from the filtrate. The mycelia were extracted three times with an 80% volume of aqueous acetone. The acetone solution was concentrated under reduced pressure to give an aqueous solution. The aqueous solution was extracted three times with an equivalent volume of ethyl acetate (EtOAc), while the filtrate was extracted three times with an equivalent volume of EtOAc. All EtOAc extracts were combined and concentrated under vacuum to give 240 g of crude gum.

3.4. Purification

The crude gum (240 g) was separated into ten fractions (Fr1–Fr10) on a silica gel VLC column using a stepwise gradient elution of petroleum ether (PE), PE-CH$_2$Cl$_2$ (1:1–0:1) followed by CH$_2$Cl$_2$-MeOH (1:1). Fr9 (26 g) was fractionated on Sephadex LH-20, eluted with CH$_2$Cl$_2$-MeOH (1:1), to obtain three subfractions (Fr9.1–Fr9.3). Fr9.2 (8 g) was further separated into five subfractions (Fr9.2.1–Fr9.2.5) by VLC on the RP-18 column using a stepwise gradient elution of MeOH-H$_2$O (9:1–1:1), among which the elution of 40% MeOH–H$_2$O gave compound 7 (9.2 mg). Compounds 1 (6.2 mg, $t_R$ 11.8 min) and 2 (32 mg, $t_R$ 18.7 min) were obtained from Fr9.2.2 (1.7 g) by semipreparative HPLC over an ODS column eluting with 15% MeCN-H$_2$O containing 0.5‰ Et$_3$N. Fr7 (12 g) was fractionated on Sephadex LH-20, eluted with MeOH-CH$_2$Cl$_2$ (1:1), to obtain four subfractions (Fr7.1–Fr7.4). Fr7.4 (3.3 g) was further purified by semipreparative HPLC over an ODS column eluting with 40% MeCN-H$_2$O containing 0.5‰ TFA (trifluoroacetic acid) to yield compound 4 (7.6 mg, $t_R$ 16.5 min). Fr7.3 (1.3 g) was fractionated into four subfractions (Fr7.3.1–Fr7.3.5) on a RP-18 column using a stepwise gradient elution of MeOH-H$_2$O (1:9–2:3). Fr7.3.2 (300 mg) was further separated by semipreparative HPLC on an ODS column eluted with 20% MeCN-H$_2$O to yield compound 3 (3.1 mg, $t_R$ 7.8 min). Fr6 (17.6 g) was further fractionated on Sephadex LH-20 eluted with MeOH-CH$_2$Cl$_2$ (1:1) to afford four subfractions (Fr6.1–Fr6.4). Fr6.2 (1.1 g) was further separated by semipreparative HPLC on an ODS column eluted with 40% MeCN-H$_2$O containing 0.5‰ TFA to yield compound 6 (16.3 mg, $t_R$ 15 min), while compound 5 (860 mg, $t_R$ 17.0 min) was purified from Fr6.4 (9 g) by semipreparative HPLC on an ODS column eluted with 65% MeCN-H$_2$O. Pure compounds 5a (8.8 mg, $t_R$ 39 min), 5b (5.4 mg, $t_R$ 42 min), 5c (7.3 mg, $t_R$ 44 min) and 5d (6.8 mg, $t_R$ 46 min) were obtained from compound 5 by a careful separation on an ODS column eluted with 50% MeOH-H$_2$O.

3.5. The Preparation of Acetonide (5e) for Relative Configuration

According to our procedure [16], compound 5a (5 mg) in acetone (3 mL) was added to the mixture of 2,2-dimethoxypropane (1 mL), pyridinium p-toluenesulfonate (PPTS, 26 mg) and N,N-dimethylformamide (DMF, 1 mL). The resulting solution was stirred at room temperature (rt) for 12 h, and then 5 mL of H$_2$O was added. The reaction solution was extracted with 15mL of CH$_2$Cl$_2$, and the organic phase was concentrated under reduced pressure. The residue was purified by semipreparative HPLC (95% MeOH-H$_2$O) to yield the acetonide 5e (3.4 mg, $t_R$ 5.7 min). Its structure was identified by ESIMS (Figure S65) and NMR data (Tables 3 and 4, Figures 4 and S66–S71).
3.6. The Induced ECD Spectra of Compounds 2, 5c, and 5d for Absolute Configuration

According to a published procedure [16, 17], analytical pure DMSO was dried with 4 Å molecular sieves and was used to prepare 0.6 mg/mL of Mo2(OAc)4 solution. To three pieces of this solution (each 1 mL, 1.40 μmol), compounds 2 (0.5 mg, 1.86 μmol), 5c (0.8 mg, 1.90 μmol), and 5d (0.8 mg, 1.90 μmol) were respectively added and the first ECD spectra of the mixtures were recorded immediately. Then, ECD spectra were continuously recorded every 10 min until stationary. The inherent ECD spectrum was subtracted. The observed signs of the diagnostic bands in the region of λmax 300–400 nm in the induced ECD spectra were correlated to the absolute configuration of the ortho-diol moiety.

(2R,3R,5S,9R,10S)-2,3,9,11-Tetrahydropyranylimid-7-en-6-one (ustusol F, 1): colorless oil; [α]23 D −56.0 (c 0.11, MeOH); UV (MeOH) λmax (log e) 232 (0.82) nm; ECD (1.76 mM, MeOH) λmax (Ae) 336 (+8.4), 271 (−3.2), 240 (−41.3), 215 (−12.8) nm; IR (KBr) νmax 3399, 2959, 1663, 1439, 1384, 1243, 1062, 1027 cm−1; 1H and 13C NMR see Table 1; HRESIMS m/z 285.1694 [M+H]⁺ (calcd for C15H24O5, 285.1697), or 283.1547 [M−H]⁻ (calcd for C15H22O5, 283.1551).

(2R,3R,5S,9R,10S),23,11-Tetrahydroxymid-7-en-6-one (9-deoxyustusol F, 2); yellow oil; [α]23 D −56 (c 0.06, MeOH); UV (MeOH) λmax (log e) 238 (1.65) nm; ECD (1.87 mM, MeOH) λmax (Ae) 334 (+6.8), 264 (−1.3), 240 (−18.3), 220 (−14.3) nm; IR (KBr) νmax 3398, 2942, 1659, 1440, 1382, 1237, 1152, 1060, 983 cm−1; 1H and 13C NMR see Table 1; HRESIMS m/z 269.1751 [M+H]⁺ (calcd for C14H21O5, 269.1747).

(3S,5R,9R,10R)-3,11,12-Tetrahydroxymid-7-en-6-one (ustusol G, 3); colorless oil; [α]23 D −71 (c 0.04, MeOH); UV (MeOH) λmax (log e) 240 (1.60) nm; ECD (1.87 mM, MeOH) λmax (Ae) 335 (+10.6), 265 (−2.6), 241 (−19.1), 205 (−71.7) nm; 1H and 13C NMR see Table 1; HRESIMS m/z 269.1750 [M+H]⁺ (calcd for C14H21O5, 269.1747).

(5S,6R,9S,10S,11R,2′E,4′E)-6-(11-Deoxy-11-hydroxyxrilbactone A-6-yl)-5-carboxypenta-2,4-dienoate (ustusolate H, 4); colorless solid; [α]23 D −96 (c 0.2, MeOH); UV (MeOH) λmax (log e) 264 (1.54) nm; ECD (0.64 mM, MeOH) λmax (Ae) 264 (−6.2), 232 (−3.3), 205 (−11.1) nm; IR (KBr) νmax 3434, 2953, 2926, 2856, 1684, 1640, 1460, 1398, 1310, 1260, 1208, 1136, 1028, 913 cm−1; 1H and 13C NMR see Table 2; HRESIMS m/z 391.1762 [M+H]⁺ (calcd for C21H27O7, 391.1762).

(5S,6R,9S,10S)-Strobilactone A-6-yl (2′E,4′E)-6,7-dihydroxyocta-2,4-dienoate (ustusolate I, 5); light yellow oil; UV (MeOH) λmax (log e) 265 (4.15) nm; 1H NMR (DMSO-d6, 500 MHz) δH 1.83 (d, J = 13.6 Hz, 1H, H-1a), 1.95 (dd, J = 4.4, 13.6 Hz, 1H, H-1β); 1.59 (m, 1H, H-2a), 1.47 (m, 1H, H-2β); 1.20 (td, J = 3.2, 13.1 Hz, 1H, H-3α), 1.34 (d, J = 12.3 Hz, 1H, H-3β); 2.00 (d, J = 5.0 Hz, 1H, H-5), 5.59 (brs, 1H, H-6); 5.79 (brs, 1H, H-7); 4.88 (dt, J = 2.3, 12.6 Hz, 1H, H-12a), 4.78 (d, J = 12.6 Hz, 1H, H-12β); 1.06 (s, 3H, H-13); 0.92 (s, 3H, H-14); 1.07 (s, 3H, H-15); 5.94 (d, J = 15.3 Hz, 1H, H-12); 7.20/7.23 (m, 1H, H-3′); 6.40/6.44 (m, 1H, H-4′); 6.30/6.34 (m, 1H, H-5′); 3.85/3.97 (m, 1H, H-6′); 3.49/3.56 (m, 1H, H-7′); 0.94/1.02 (d, J = 6.2 Hz, 3H, H-8′); 5.02 (brs, 1H, HO-6′); 4.61/4.66 (brs, 1H, HO-7′); 13C NMR (DMSO-d6,125 MHz) δC 29.6 (CH2-2, C-1), 17.5 (CH2-2, C-3), 44.5 (CH2, C-4), 44.2 (CH, C-5), 65.8 (CH, C-6), 121.4 (CH, C-7), 136.6 (C, C-8), 73.2 (C, C-9), 37.3 (C, C-10), 174.4 (C, C-11), 68.3 (CH2, C-12), 18.3 (CH3, C-13), 32.2 (CH2-C14), 24.4 (CH3-C15), 165.5/165.5/165.4/165.7 (C, C-1′), 120.3/120.9/119.5/119.9 (CH, C-2′), 145.4/145.37/145.34/145.26 (CH, C-3′), 127.54/127.35/127.16/126.98 (CH, C-4′), 146.18/146.12/145.48/145.45 (CH, C-5′), 75.16/75.00/74.64/74.46 (CH, C-6′), 69.64/69.62/69.33/69.32 (CH, C-7′), and 19.34/19.26/18.26/18.24 (CH3, C-8′); ESIMS peak at m/z 419.1 for [M−H]⁻ and m/z 464.9 for [M + HCO2]⁺ (C21H22O7).

(2′E,4′E,6′,7′-erythro)-Ustusolate I (5a): light yellow oil; [α]23 D −35 (c 0.30, MeOH); UV (MeOH) λmax (log e) 268 (4.15) nm; ECD (0.60 mM, MeOH) λmax (Ae) 255 (−8.3), 236 (−8.9), 208 (−21.2) nm; 1H and 13C NMR see Tables 3 and 4; ESIMS m/z 421.2 [M+H]⁺ (C23H32O7).

(2′E,4′E,ent-6′,7′-erythro)-Ustusolate I (5b): light yellow oil; [α]23 D −42 (c 0.30, MeOH); UV (MeOH) λmax (log e) 261 (4.39) nm; ECD (0.60 mM, MeOH) λmax (Ae) 256 (−11.0), 234 (−8.9), 209 (−21.4) nm; 1H and 13C NMR see Tables 3 and 4; ESIMS m/z 421.2 [M+H]⁺ (C23H32O7).
(2'E,4'E,6'R,7'R)-Ustusolate I (5c): light yellow oil; [α]_D^23 = −105 (c 0.30, MeOH); UV (MeOH) _λ_ max (log ε) 261 (4.42) nm; ECD (0.60 mM, MeOH) _λ_ max (Δε) 256 (−8.6), 234 (−8.5), 208 (−22.9) nm; 1H and 13C NMR see Tables 3 and 4; ESIMS m/z 421.2 [M+H]^+ (C_{23}H_{32}O_7).

(2'E,4'E,6'S,7'S)-Ustusolate I (5d): light yellow oil; [α]_D^23 = −79 (c 0.29, MeO); UV (MeOH) _λ_ max (log ε) 262 (4.36) nm; ECD (0.60 mM, MeOH) _λ_ max (Δε) 259 (−10.4), 234 (−8.2), 208 (−18.9) nm; 1H and 13C NMR see Tables 3 and 4; ESIMS m/z 421.2 [M+H]^+ (C_{23}H_{32}O_7).

1. Author Contributions:

   A. T.Z. performed the cultivation and extraction of ustusolate analogues (5c).
   B. J.F. prepared the draft of the manuscript.
   C. F. isolated and identified the constitution of the compounds.
   D. CAL-62 and MG-63 tumor cells with the IC_{50} values of 16.3 and 10.1 μM, respectively, while the purified compounds 5a–5d didn’t show activity.

2. Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/md20070408/s1, the HRESIMS of compounds 1–5 and the NMR spectra of compounds 1–7, the analysis for the bacteriostatic activities and the cytotoxic activities, the HPLC separation, and purification profiles of 5a–5d.

3. Informed Consent Statement: This research is not involving humans or animals.

4. Data Availability Statement: This research is not involving humans or animals.

5. Conflicts of Interest: The authors declare no conflict of interest.
References

1. Cragg, G.M.; Grothaus, P.G.; Newman, D.J. New horizons for old drugs and drug leads. *J. Nat. Prod.* 2014, 77, 703–723. [CrossRef] [PubMed]

2. Meng, L.H.; Li, X.M.; Li, H.L.; Wang, B.G. Chermebilaenes A and B, new bioactive meroterpenoids from co-cultures of marine-derived isolates of *Penicillium bilaiae* MA-267 and *Penicillium chermesinum* EN-480. *Mar. Drugs* 2020, 18, 339. [CrossRef] [PubMed]

3. Bugni, T.S.; Ireland, C.M. Marine-derived fungi: A chemically and biologically diverse group of microorganisms. *Nat. Prod. Rep.* 2004, 21, 143–163. [CrossRef] [PubMed]

4. Lu, Z.; Wang, Y.; Miao, C.; Liu, P.; Hong, K.; Zhu, W. Sesquiterpenoids and benzofuranoids from the marine-derived fungus *Aspergillus ustus* 094102. *J. Nat. Prod.* 2009, 72, 1761–1767. [CrossRef] [PubMed]

5. Zhu, T.; Lu, Z.; Fan, J.; Wang, L.; Zhu, G.; Wang, Y.; Li, X.; Hong, K.; Piyachaturawat, P.; Chairoungdua, A.; et al. Ophiobolins from the mangrove fungus *Aspergillus ustus*. *J. Nat. Prod.* 2018, 81, 2–9. [CrossRef] [PubMed]

6. Shiono, Y.; Hiramatsu, F.; Murayama, T.; Koseki, T.; Funakoshi, T.; Ueda, K.; Yasuda, H. Two drimane-type sesquiterpenes, strobilactones a and b, from the liquid culture of the edible mushroom *Strobilurus ohshimae*. *Z. Naturforsch.* 2007, 62b, 1585–1589. [CrossRef]

7. Liu, H.; Edrada-Ebel, R.; Ebel, R.; Wang, Y.; Schulz, B.; Draeger, S.; Werner, E.G.M.; Wray, V.; Lin, W.; Proksch, P. Drimane sesquiterpenoids from the fungus *Aspergillus ustus* isolated from the marine sponge *Suberites domuncula*. *J. Nat. Prod.* 2009, 72, 1585–1588. [CrossRef] [PubMed]

8. Zhou, H.; Zhu, T.; Cai, S.; Gu, Q.; Li, D. Drimane sesquiterpenoids from the mangrove-derived fungus *Aspergillus ustus*. *Chem. Pharm. Bull.* 2011, 59, 762–766. [CrossRef] [PubMed]

9. Neuhaus, G.F.; Loesgen, S. Antibacterial drimane sesquiterpenes from *Aspergillus ustus*. *J. Nat. Prod.* 2021, 84, 37–45. [CrossRef] [PubMed]

10. Hayes, M.A.; Wrigley, S.K.; Chetland, I.; Reynolds, E.E.; Ainsworth, A.M.; Renno, D.V.; Latif, M.A.; Cheng, X.M.; Hupe, D.J.; Peter, C.; et al. Novel drimane sesquiterpene esters from *Aspergillus ustus var. pseudodeflectus* with endothelin receptor binding activity. *J. Antibiot.* 1996, 49, 505–512.

11. Mi, J.F.; Xu, R.S.; Yang, Y.P.; Yang, P.M. Studies on circular dichroism of diterpenoids from *Mallotus anomalus* and Sesquiterpenoid tussilagone. *Acta Pharm. Sin.* 1993, 28, 105–109.

12. Jiang, Y.; Liu, Y.; Guo, Q.; Jiang, Z.; Xu, C.; Zhu, C.; Yang, Y.; Lin, S.; Shi, J. Acetylenes and fatty acids from *Codonopsis pilosula*. *Acta Pharm. Sin. B* 2015, 5, 215–222. [CrossRef] [PubMed]

13. Ebel, R. Terpenes from marine-derived fungi. *Mar. Drugs* 2010, 8, 2340–2368. [CrossRef] [PubMed]

14. Garlaschelli, L.; Vidari, G. Synthetic studies on biologically active natural compounds. Part I. Stereosepecific transformation of uvidin A into (−)-cinnamodial. *Tetrahedron* 1989, 45, 7371–7378. [CrossRef]

15. Bari, L.D.; Pescitelli, G.; Pratelli, C.; Pini, D.; Salvadori, P. Determination of absolute configuration of acyclic 1,2-diols with Mo₂(OAc)₄. 1. Snatzke’s method revisited. *J. Org. Chem.* 2001, 66, 4819–4825. [CrossRef] [PubMed]

16. Fan, Y.; Wang, Y.; Liu, P.; Zhu, T.; Zhu, W. Indole-diterpenoids with anti-H1N1 activity from the aciduric fungus *Penicillium camemberti* OUCMDZ-1492. *J. Nat. Prod.* 2013, 76, 1328–1336. [CrossRef] [PubMed]

17. Wang, L.; Zhu, W. Versicolactones A and B: Total synthesis and structure revision. *Tetrahedron Lett.* 2013, 54, 6729–6731. [CrossRef]

18. Tominaga, H.; Ishiyama, M.; Ohseoto, F.; Sasamoto, K.; Hamamoto, T.; Suzuki, T.; Watanabe, M. A water-soluble tetrazolium salt useful for colorimetric cell viability assay. *Anal. Commun.* 1999, 36, 47–50. [CrossRef]

19. Wang, D.; Wang, C.; Gui, P.; Liu, H.; Khalaf, S.M.H.; Elsayed, E.A.; Wadaan, M.A.M.; Hozzein, W.N.; Zhu, W. Identification, bioactivity, and productivity of *Actinomycins* from the marine-derived *Streptomyces heliomycini*. *Front. Microbiol.* 2017, 8, 1147. [CrossRef] [PubMed]