Sesquiterpenoids and Benzofuranoids from the Marine-Derived Fungus *Aspergillus ustus* 094102

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Eight drimane sesquiterpenes (**1**−**8**), six isochromane derivatives (**9**−**14**), and three known compounds, daldinin B (**15**), 9α-hydroxy-6*β*-[(2*E*,4*E*,6*E*)-octa-2,4,6-trienoyloxy]-5α-drim-7-en-11,12-olide (**16**), and pergillin (**17**), were isolated from the EtOAc extract of the marine-derived fungus *Aspergillus ustus* 094102. The structures of the new compounds were elucidated on the basis of spectroscopic analysis. The cytotoxic effects on A549 and HL-60 cell lines were evaluated by SRB and MTT methods. Ustusorane E (**13**) showed significant cytotoxicity against HL-60 cells with an IC_{50} value of 0.13 μM. Ustusolates C (**6**) and E (**8**) exhibited moderate cytotoxicity against A549 and HL-60 cells with IC_{50} values of 10.5 and 9.0 μM, respectively, and ustusolate A (**4**) showed weak cytotoxicity against HL-60 and A549 cells with IC_{50} values of 20.6 and 30.0 μM, respectively.

As a part of our ongoing search for active compounds from microorganisms isolated from unusual or specialized ecological niches,[1−3] we recently have focused on the microorganisms from mangrove plants. Due to the special living environment of low-wave-energy tidal mudflats, mangrove plants have been considered as a source of unique compounds possessing physiological activities. Investigation of the secondary metabolites of microorganisms collected from mangrove plants may also increase the chance of finding novel active compounds. Our study focused on the chemical composition of the fungus *Aspergillus ustus* 094102, isolated from the rhizosphere soil of the mangrove plant *Bruguiera gymnorrhiza* grown in Wenchang, Hainan Province of China. The chemical constituents of *A. ustus* have been studied by many groups. Metabolites of this species include meroterpenoid mycotoxins,[4−6] isochromane derivatives,[7−9] drimane sesquiterpene esters,[10] and ophiobolins.[11] Many of them exhibited interesting biological activities, such as plant growth inhibition[7] and inhibition of endothelin-type B receptors.[10] Further chemical study of this new strain was still worth conducting because the fermentation products showed cytotoxicity against B16 cell lines and antifungal activity against *Candida albicans*. Chemical studies resulted in the identification of 14 new compounds including three drimane sesquiterpenes [ustusols A−C (**1**−**3**)], five drimane sesquiterpene esters [ustusolates A−E (**4**−**8**)], six benzofuran derivatives [ustusoranes A−F (**9**−**14**)], and three known compounds [daldinin B (**15**),[12] 9α-hydroxy-6*β*-[(2*E*,4*E*,6*E*)-octa-2,4,6-trienoyloxy]-5α-drim-7-en-11,12-olide (**16**),[10] and pergillin (**17**)[13]] from the fermentation broth of *A. ustus* 094102. Among them, compounds **7**, **11**, and **12** are all methyl acetals and might be isolation artifacts formed by the reaction of the corresponding aldehyde (**8**) or hemiacetals with MeOH. Only seven drimane sesquiterpene esters esterified at C-6 have been reported previously.[10,14]

Results and Discussion

Ustusol A (**1**), obtained as a white solid, was assigned the molecular formula $C_{15}H_{24}O_4$ from HRESIMS (*m/z* 269.1762 [M + H]$^+$), which was consistent with four degrees of unsaturation. The IR spectrum showed the presence of hydroxy groups (3520, 3440, 3380 cm^{-1}) and a conjugated carbonyl group (1660 cm^{-1}). The ^{1}H NMR spectrum of **1** (Table 1) revealed four methyls including three aliphatic singlet methyls (δ_H 0.99, 1.13, and 1.02) and an olefinic methyl (δ_H 1.97), an olefinic proton (δ_H 5.60), an oxygenated methine (δ_H 2.93), and three methylenes (δ_H 1.99/1.48, 1.53, 3.64/3.52). The ^{13}C NMR (DEPT) spectrum exhibited 15 signals corresponding to four methyls, three methylenes, three methines, a ketone carbonyl, and four quaternary carbons. These NMR data revealed that **1** was a drimane sesquiterpene.[10] The ^{1}H−^{1}H COSY experiment disclosed two structural moieties, −CH_2CH_2CHOH and −CH_2OH, corresponding to the C-1, C-2, C-3(OH), and C-11(OH) fragments. The HMBC correlations between H-1 and both C-5 and C-10; between H-5 and C-4, C-10, C-13, and C-14; between H-7 and C-5, C-9, and C-12, and between H-11 and both C-9 and C-10 enabled the specific connection of groups that were not readily identifiable as parts of spin systems from the ^{1}H−^{1}H COSY experiment. Thus, the constitution of **1** was established as 3,9,11-trihydroxydrim-7-en-6-one. NOESY correlations between H-13 and H-1b$_{(eq)}$, and between H-5 and H-1a$_{(ax)}$, indicated a *trans*-fused decalin nucleus. The correlations of H-3 with H-5 and H-1a$_{(ax)}$, of OH-9 with H-5 and H-1a$_{(ax)}$, and of H-13 with H-11 revealed *cis*-configurations between H-5 and OH-9 and between OH-3 and both OH-11 and CH_3-13. Thus, the relative configuration of **1** was elucidated as 3*β*,9α,11-trihydroxy-5α-drim-7-en-6-one (Figure 1). The absolute configuration of **1** was determined by its CD spectrum. On the basis of the octant rule for cyclohexenones,[15] the positive Cotton effect at 328 nm ($\Delta\varepsilon_{max}$ +20.3) for n→π* and the negative Cotton effect at 235 nm ($\Delta\varepsilon_{max}$ −83.2) for π→π* indicated that the absolute configuration of **1** was (3*S*,5*S*,9*R*,10*S*), consistent with the core configuration of other drimane sesquiterpene analogues, such as RES-1149-1[14] and 9α,11-dihydroxy-6-oxodrim-7-ene,[16] whose absolute configurations have been established by chemical synthesis. Therefore, the structure of ustusol A (**1**) was determined as (3*S*,5*S*,9*R*,10*S*)-3,9,11-trihydroxydrim-7-en-6-one.

Ustusol B (**2**) was obtained as a colorless solid. The molecular formula of **2** was assigned as $C_{15}H_{24}O_4$ from the HRFABMS (*m/z* 291.1577 [M + Na]$^+$), which was the same as **1**. The IR spectrum also showed the presence of hydroxy groups (3400, 3320 cm^{-1}) and a conjugated carbonyl group (1658 cm^{-1}). Except for those of the C_1−C_2−C_3 segment, the 1D NMR data of **2** (Table 1) revealed considerable similarity to **1**, implying that they shared the same drimane sesquiterpene skeleton. The ^{1}H−^{1}H COSY correlations of H-2 with H-3 and H-1 and of the OH-2 with H-2 indicated a 2-substituted hydroxy group, which was further confirmed by the key HMBC correlations between OH-2 and C-1, C-2, and C-3. So the constitution of **2** was determined as 2,9,11-trihydroxydrim-7-

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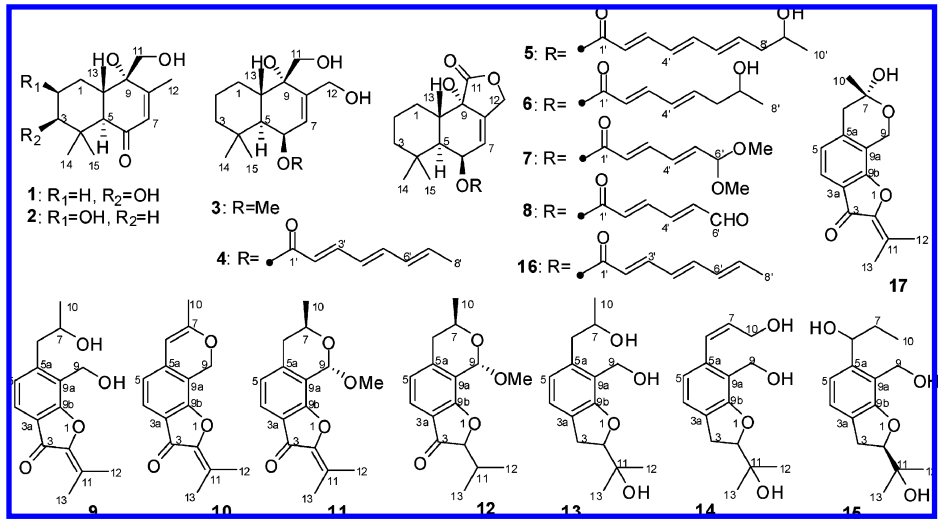
Chart 1



en-6-one. The same relative configuration as **1** was deduced from the NOESY correlations of H-15 with OH-2 and H-13, of H-13 with H-12, and of H-5 with H-14 and OH-9. The CD spectra of **2** showed the same Cotton effect as that of **1** at 328 nm ($\Delta\varepsilon_{max}$ +16.2) and 235 nm ($\Delta\varepsilon_{max}$ −71.5), indicating the same core configuration. Thus, the structure of ustusol B (**2**) was determined as (2*R*,5*S*,9*R*,10*S*)-2,9,11-trihydroxydrim-7-en-6-one.

Ustusol C (**3**) was obtained as a colorless solid. The pseudomolecular ion at *m*/*z* 307.1891 $[M + Na]^+$ in the HRESIMS spectrum was assigned to the molecular formula $C_{16}H_{28}O_4$, corresponding to three degrees of unsaturation. Its ^1H NMR spectrum was very similar to that of 12-hydroxy-6-*epi*-albrassitriol from *Aspergillus* sp.,[17] with an additional methoxy signal at δ_H 3.23 and a −0.37 ppm upfield shift of H-6. An additional methoxy signal at δ_C 53.8 and a +7.3 ppm downfield shift of C-6 were observed in the ^{13}C NMR (DEPT) spectrum (Table 2). These findings combined with the key HMBC correlation from 6-OCH_3 to C-6 established a planar structure for **3** consistent with 6-*O*-methyl-12-hydroxyalbrassitriol. Ustusolate A (**4**) was obtained as a colorless oil. HRESIMS gave an exact mass of *m*/*z* 391.2485 for $[M + H]^+$, suggesting a molecular formula of $C_{23}H_{34}O_5$. Its IR spectrum showed absorption bands for a hydroxy at 3431 cm^{-1} and a conjugated ester group at 1705 cm^{-1}. Inspection of the ^1H and ^{13}C NMR data of **4** revealed that it shared the same drimane sesquiterpene skeleton as **3**. Its ^1H NMR spectrum (Table 1) showed six additional *trans*-coupled olefinic protons (δ_H 5.89, br d, J = 15.1; 7.19, dd, J = 15.1, 11.5; 6.34, dd, J = 14.7, 11.5; 6.68, dd, J = 14.7, 11.0; 6.20, dd, J = 15.1, 11.0; 6.01, dq, J = 15.1, 6.9) and an additional doublet methyl group (δ_H 1.79, br d, J = 6.9) coupled with the olefinic proton at δ_H 6.01. The $^1H-^1H$ COSY experiment disclosed that these additional protons connected together to form a CH═CH−CH═CH−CH═CH−CH_3 moiety corresponding to C-2′ through C-8′. These data along with the key HMBC correlations from H-6 and H-3′ to C-1′ confirmed the moiety is a 2*E*,4*E*,6*E*-octatrienoyloxy group, located at position 6 of the skeleton. The constitution of **4** was thus elucidated as 9,11,12-trihydroxydrim-7-en-6-yl octa-2*E*,4*E*,6*E*-trienoate. NOESY correlations between H-15 and H-13 and between H-5 and H-14 indicated a *trans*-fused decalin nucleus in both **3** and **4**. The NOESY correlations between H-13 and H-11 and between H-6 and H-5 revealed *cis*-configurations between CH_3-13, $HOCH_2$-11, and 6-OCH_3 in **3** or the 6-acyl group in **4**. Thus, the structures of ustusol C (**3**) and ustusolate A (**4**) were elucidated as 6-*O*-methyl-12-hydroxy-6-*epi*-albrassitriol and 6-*O*-(octa-2*E*,4*E*,6*E*-trienoyl)-12-hydroxy-6-*epi*-albrassitriol, respectively.

Ustusolates B−E (**5**−**8**) were all obtained as colorless oils. Their molecular formulas were determined as $C_{25}H_{34}O_6$, $C_{23}H_{32}O_6$, $C_{23}H_{32}O_7$, and $C_{21}H_{26}O_6$ from HRESIMS data at *m*/*z* 453.2265 $[M + Na]^+$, 405.2293 $[M + H]^+$, 419.2078 $[M - H]^-$, and 375.1804 $[M + H]^+$, respectively. Examination of their ^1H and ^{13}C NMR spectra (Tables 1 and 2) revealed that they shared the same drimane sesquiterpene skeleton as **16**,[10] with variation only in the fatty acyl moiety. In comparison to **16**, there were three additional signals including an oxygenated methine ($\delta_{H/C}$ 3.68/65.7), a methylene ($\delta_{H/C}$ 2.20/42.5), and an exchangeable proton (δ_H 4.58) in **5**. The downfield shifts of C-7′ (+2.6 ppm) and the doublet methyl (+5.0 ppm) suggested that the additional oxygenated methine and methylene were located between C-7′ and the doublet methyl. These data suggested a 9-hydroxydeca-2*E*,4*E*,6*E*-trienoyl group, which was further verified by the $^1H-^1H$ COSY experiment (Figure 2). The 1D NMR spectra of **6** were very similar to those of **5**, except for the absence of two olefinic methine groups, which revealed the fatty acyl moiety as a 7-hydroxyocta-2*E*,4*E*-dienoyl group. This deduction was further supported by a $^1H-^1H$ COSY experiment (Figure 2). The ^1H and ^{13}C NMR spectra of **7** showed the presences of a dimethyl acetal at $\delta_{H/C}$ 3.33/52.8 and 4.92/101.3, which was confirmed by the key HMBC correlation between the methoxy protons (δ_H 3.33, 6H, s) and the acetal carbon (δ_C 101.3, CH). The $^1H-^1H$ COSY experiment established the fragment CH═CH−CH═CH−CH. These data implied a 6,6-dimethoxyhex-2*E*,4*E*-dienoyl group. In comparison to **7**, the ^1H and ^{13}C NMR spectra (Tables 1 and 2) of **8** showed an aldehyde group at $\delta_{H/C}$ 9.68/192.8 (CH) rather than an acetal group. The $^1H-^1H$ COSY correlations of H-2′ to H-3′ and H-4′ to H-6′ (aldehyde group) through H-5′ confirmed that the fatty acyl group was 6-oxohex-2*E*,4*E*-dienoyl. The 2D NOESY experiments of ustusolates B−E (**5**−**8**) all exhibited the same correlations between H-15 and H-13, between H-14 and H-6, and between H-5 and H-6, OH-9, and H-14, indicating that they shared the same relative configurations. Thus, the structures of ustusolates B−E (**5**−**8**) were determined as 9α-hydroxy-6*β*-(9-hydroxydeca-2*E*,4*E*,6*E*-trienoyloxy)-5α-drim-7-en-11,12-olide, 9α-hydroxy-6*β*-(7-hydroxyocta-2*E*,4*E*-dienoyloxy)-5α-drim-7-en-11,12-olide, 9α-hydroxy-6*β*-(6,6-dimethoxyhex-2*E*,4*E*-dienoyloxy)-5α-drim-7-en-11,12-olide, and 9α-hydroxy-6*β*-(6-oxohex-2*E*,4*E*-dienoyloxy)-5α-drim-7-en-11,12-olide, respectively.

Ustusorane A (**9**) was obtained as a pale yellow solid. Its HRESIMS gave an exact mass of *m*/*z* 263.1275 for $[M + H]^+$, suggesting a molecular formula of $C_{15}H_{18}O_4$. The IR spectrum showed the presence of hydroxy (3380 cm^{-1}) and conjugated carbonyl (1690 cm^{-1}) groups. Examination of the ^1H NMR spectrum (Table 1) of **9** indicated unambiguously the presence of a 1,2,3,4-tetrasubstituted benzene (δ_H 7.55, d, J = 7.8; δ_H 6.95, d, J = 7.8), one oxygenated methylene (δ_H 4.72, 4.91), one oxygenated methine (δ_H 4.08), one methylene (δ_H 2.90, 2.95), two aromatic or olefinic methyls (δ_H 2.11, δ_H 2.35), and one doublet methyl (δ_H

Table 1. ^{1}H NMR Data for Compounds **1**−**14** (600 MHz, DMSO-d_6, TMS, δ in ppm, J in Hz)

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**[a]
1	1.99, m;	1.70, m	1.62, dt (12.8, 3.3);	1.86, dt (13.3, 3.7);	1.96, dd (14.9, 4.4);	1.96, dd (13.7, 4.4);	2.09, br d (12.2);
	1.48, m		1.45, m	1.43, m	1.83, br d (14.9)	1.84, br d (13.7)	1.72, m
2	1.53, m	3.71, m	1.48, m; 1.38, m	1.60, m; 1.44, m	1.60, m; 1.48, m	1.61, m; 1.47, m	1.75, m; 1.60, m
3	2.93, ddd (10.4, 9.9, 4.9)	1.51, m;	1.29, br d (12.8)	1.28, m; 1.17, m	1.34, br d (13.2)	1.34, br d (13.2)	1.42, br d (12.8)
		0.96, br.t (12.0)	1.14, dt (12.8, 3.3)		1.21, dt (13.2, 2.8)	1.21, dt (13.2, 2.8)	1.32, m
4							
5	2.75, s	2.71, s	1.88, d (10.3)	1.97, d (4.6)	2.01, d (5.0)	2.01, d (4.9)	2.04, d (4.9)
6			3.73, dd (10.4, 1.3)	5.54, "t" like (4.4)	5.58, br s	5.59, br s	5.73, br s
7	5.60, d (1.1)	5.62, d (1.1)	5.83, br s	5.77, d (5.0)	5.80, br s	5.79, br s	5.90, br s
9							
10							
11	3.64, dd (11.5, 4.9)	3.66, dd (11.5, 4.8)	3.49, dd (11.8, 6.0)	3.52, dd (11.5, 5.9)			
	3.52, dd (11.5, 4.4)	0.96, dd (11.5, 4.8)	3.42, dd (11.8, 4.4)	3.45, dd, (11.5, 4.1)			
12	1.97, d (1.1)	1.98, br s	4.07, dd (14.1, 5.1)	4.13, dd (13.8, 5.0)	4.80, br d (12.6)	4.79, br d (12.6)	4.74, br d (12.7)
			4.10, dd (14.1, 5.1)	4.15, dd (13.8, 5.0)	4.88, dt (12.6, 2.4)	4.87, dt (12.6, 2.2)	4.97, dt (12.7, 2.4)
13	0.99, s	1.08, s	0.92, s	1.17, s	1.06, s	1.06, s	1.19, s
14	1.13, s	1.15, s	1.05, s	0.91, s	0.92, s	0.92, s	1.00, s
15	1.02, s	1.03, s	1.00, s	1.06, s	1.07, s	1.07, s	1.12, s
2′				5.89, br d (15.1)	5.93, br d (14.9)	5.88, br d (15.4)	5.95, br d (15.4)
3′				7.19, dd (15.1, 11.5)	7.25, dd (14.9, 11.0)	7.19, dd (15.4, 10.4)	7.25, dd (15.4, 11.0)
4′				6.34, dd (14.7, 11.5)	6.38, dd (14.9, 11.0)	6.31, dd (15.4, 10.4)	6.50, dd (15.4, 11.0)
5′				6.68, dd (14.7, 11.0)	6.73, dd (14.9, 11.0)	6.27, dd (15.4, 7.1)	6.02, dd (15.4, 4.4)
6′				6.20, dd (15.1, 11.0)	6.20, dd (14.9, 11.0)	2.22, m	4.92, d (4.4)
7′				6.01, dq (15.1, 6.9)	6.03, dt (14.9, 7.7)	3.70, m	
8′				1.79, d (6.9)	2.20, m	1.05, d (6.6)	
9′					3.68, m		
10′					1.04, d (6.0)		
−OCH$_3$			3.23, s				3.33, s, 6H
2-OH		4.41, d (5.1)					
3-OH	4.39, d (5.0)						
7-OH							
9-OH	4.94, s	5.02, s	4.24, s	4.40, s	6.30, s	6.30, s	
10-OH							
11-OH	4.81, dd (4.9, 4.4)	4.86, t (4.8)	4.64, (5.1)	4.63, t (5.0)			
12-OH			4.89, t (5.1)	4.85, t (5.0)			
7′-OH						4.63, d (4.4)	
9′-OH					4.58, d (4.4)		

position	**8**[a]	**9**[a]	**10**[b]	**11**[a]	**12**[a]	**13**	**14**
1	2.11, br d (12.8);						
	1.73, m						
2	1.74, m; 1.62, m				4.53, d (3.7)	4.50, m	4.53, dd (9.1, 8.0)
3	1.44, br d (12.8)					3.08, dd (15.8, 9.1)	3.10, dd (16.1, 9.3)
	1.33, br t (12.8)					3.12, dd (15.8, 7.8)	3.14, dd (16.1, 7.9)
4		7.55, d (7.8)	7.47, d (7.7)	7.60, d (8.0)	7.51, d (7.7)	6.98, d (7.7)	7.04, d (7.3)
5	2.07, d (4.7)	6.95, d (7.8)	6.77, d (7.7)	6.86, d (8.0)	6.80, d (7.7)	6.62, d (7.7)	6.54, d (7.3)
6	5.77, br s	2.95, dd (13.7, 9.2)	5.85, s	2.77, dd (17.2, 3.3)	2.77, dd (17.6, 3.3)	2.74, dd (13.2, 7.3);	6.68, br d (11.8)
		2.90, dt (13.7, 3.0)		2.70, dd (17.2, 11.0)	2.69, dd (17.6, 11.0)	2.60, dd (13.2, 5.5)	
7	5.92, br s	4.08, m		4.32, ddq (11.0, 6.2, 3.7)	4.33, ddq (11.0, 6.2, 3.3)	3.78, m	5.78, dt (11.8, 6.6)
9		4.91, br d (12.4)	5.28, s	5.71, s	5.67, s	4.48, dd (11.4, 4.8)	4.42, dd (11.3, 5.3)
		4.72, dd (12.4, 2.3)				4.42, dd (11.4, 4.0)	4.39, dd (11.3, 5.3)
10		1.37, d (6.4)	1.95, s	1.40, d (6.2)	1.40, d (6.2)	1.07, d (7.2)	4.06, ddd (6.6, 5.3, 1.5)
11					2.36, dh (3.7, 7.0)		
12	4.74, br d (12.4)	2.11, s	2.07, s	2.12, s	0.87, d (6.6)	1.12, s	1.12, s
	4.98, dt (12.4, 2.4)						
13	1.20, s	2.35, s	2.30, s	2.36, s	1.15, d (7.0)	1.12, s	1.13, s
14	1.01, s						
15	1.13, s						
2′	6.31, br d (15.4)						
3′	7.41, dd (15.4, 11.4)						
4′	7.18, dd (15.4, 11.4)						
5′	6.45, dd (15.4, 7.7)						
6′	9.68, d (7.7)						
7′							
8′							
9′							
10′							
−OCH$_3$				3.63, s	3.61, s		
2-OH							
3-OH							
7-OH						4.78, d (5.8)	
9-OH						4.72, dd (4.8, 4.0)	4.66, t (5.1)
10-OH							4.75, t (5.3)
11-OH							
12-OH							
7′-OH							
9′-OH							

[a] Recorded in $CDCl_3$. [b] Recorded in acetone-d_6.

1.37, $J = 6.4$). The ^{13}C NMR spectrum (Table 2) showed 15 carbon resonances including a conjugated carbonyl (δ_C 183.7), six aromatic carbons, two olefinic carbons, two methylene carbons, a methine carbon, and three methyl carbons. With the exception of an oxygenated methine substitution for a hemiketal carbon, these spectroscopic data were close to those of pergillin (**17**),[13] showing

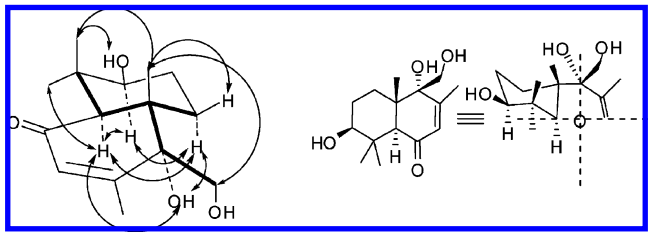


Figure 1. NOESY correlations of compound **1** and the octant rule for the cyclohexenone.

that they are analogues. In consideration of the one unsaturation less than pergillin, ustusorane A (**9**) could be a ring-opened derivative of pergillin (**17**). This presumption was further confirmed by $^1H-^1H$ COSY correlations of H-7 with H-6, H-10, and OH-7 and of H-7 with OH-7, and the key HMBC correlations from H-6 to C-7, C-5, and C-5a and from H-9 to C-5a, C-9a, and C-9b. Thus, the structure of ustusorane A (**9**) was elucidated as 7-hydroxymethyl-6-(2-hydroxypropyl)-2-(propan-2-ylidene) benzofuran-3(2*H*)-one.

The molecular formula of ustusorane B (**10**) was deduced as $C_{15}H_{14}O_3$ from the HRESIMS at *m*/*z* 243.1019 $[M + H]^+$. The 1D NMR data (Tables 1 and 2) indicated that **10** was also an analogue of **17**. Careful comparison of both 1D NMR spectra revealed that a trisubstituted ethene moiety ($\delta_{H/C}$ 5.85/102.4, 160.2) substituted for the methylene and hemiketal carbons. Upfield shifts for C-10 and C-9a and downfield shifts for C-9 and C-5a were observed. These data combined with a mass 18 amu less than **17** reveal that ustusorane B (**10**) was the dehydrated derivative between C-6 and C-7 of pergillin (**17**), which was further verified by the key HMBC correlations of H-10 to C-6, H-6 to C-5 and C-9a, and H-9 to C-7, C-5a, and C-9b. Accordingly, ustusorane B (**10**) was thus elucidated as 7-methyl-2-(propan-2-ylidene)-2*H*-furo[3,2-*h*]isochromen-3(9*H*)-one.

The molecular formula of ustusorane C (**11**) was deduced as $C_{16}H_{18}O_4$ from the HRESIMS at *m*/*z* 275.1278 $[M + H]^+$. The 1D NMR data of **11** were very close to those of pseudodeflectusin from *A. pseudodeflectus*,[18] except for an additional methoxy ($\delta_{H/C}$ 3.63/55.74) and a downfield shift of the hemiacetal carbon. These observations combined with the key HMBC correlation of 9-OCH_3 to C-9 verified that **11** was the methyl acetal of pseudodeflectusin. Compound **11** was possibly produced from two sequential reactions during the isolation process, dehydration of pseudodeflectusin with silica gel (to form an oxonium ion) and quenching of the oxonium ion by MeOH. The same *anti*-configuration as pseudodeflectusin was deduced on the basis of the mechanistic consideration that the nucleophilic attack of MeOH on the oxonium ion should occur preferably from the less hindered side. The specific rotation of **11** ($[\alpha]^{20}_D$ +6) is of the same sign as that of natural (+)-pseudodeflectusin, whose absolute configuration had been confirmed as 7*R*,9*S* by chemical synthesis,[19] suggesting that **11** has the (7*R*,9*S*)-configuration. Thus, the structure of ustusorane C (**11**) was tentatively assigned as 9-*O*-methylpseudodeflectusin, i.e., 9*S*-methoxy-7*R*-methyl-2-(propan-2-ylidene)-6,7-dihydro-2*H*-furo[3,2-*h*]isochromen-3(9*H*)-one.

Ustusorane D (**12**) was obtained as a yellow solid. HRESIMS gave an exact mass of *m*/*z* 277.1433 for $[M + H]^+$, suggesting a molecular formula of $C_{16}H_{20}O_4$, which was H_2 more than that of **11**. The 1D NMR spectra of **12** and **11** were very similar except the exocyclic double bond in **11** was changed to a saturated bond ($\delta_{H/C}$ 4.53/90.2, 2.36/31.1) in **12**. On the basis of the same reasons as those of **11**, compound **12** had the same configuration as **11** at C-7 and C-9. So, ustusorane D (**12**) was a 2,11-dihydro derivative of ustusorane C (**11**), i.e., 2-isopropyl-9-methoxy-7-methyl-6,7-dihydro-2*H*-furo[3,2-*h*]isochromen-3(9*H*)-one.

Ustusorane E (**13**) was obtained as a colorless solid. A HRESIMS peak at *m*/*z* 289.1422 for $[M + Na]^+$ corresponded to a molecular

Table 2. ^{13}C NMR Data for Compounds **1**–**14** (150 MHz, DMSO-d_6, TMS, δ in ppm)

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**[a]	**8**[a]	**9**[a]	**10**[b]	**11**[a]	**12**[a]	**13**	**14**
1	29.6, CH_2	41.0, CH_2	32.2, CH_2	31.8, CH_2	29.6, CH_2	29.6, CH_2	30.3, CH_2	30.3, CH_2						
2	26.3, CH_2	62.4, CH	18.2, CH_2	18.2, CH_2	17.4, CH_2	17.5, CH_2	17.8, CH_2	17.7, CH_2	145.2, qC	145.3, qC	145.3, qC	90.2, CH	88.5, CH	88.6, CH
3	76.7, CH	51.7, CH_2	43.1, CH_2	44.1, CH_2	44.4, CH_2	44.5, CH_2	44.8, CH_2	44.8, CH_2	183.7, qC	182.7, qC	183.3, qC	201.1, qC	30.1, CH_2	30.1, CH_2
4	37.1, qC	33.4, qC	32.8, qC	33.3, qC	33.3, qC	33.3, qC	33.9, qC	33.9, qC	123.6, CH	124.4, CH	123.7, CH	123.5, CH	123.3, CH	123.1, CH
5	55.3, CH	54.7, CH	45.7, CH	44.7, CH	44.2, CH	44.2, CH	44.8, CH	44.8, CH	124.7, CH	118.2, CH	122.6, CH	122.1, CH	121.9, CH	120.9, CH
6	199.5, qC	199.6, qC	77.1, CH	66.2, CH	65.7, CH	65.8, CH	66.6, CH	67.4, CH	41.7, CH_2	102.4, CH	35.9, CH_2	35.9, CH_2	41.5, CH_2	127.1, CH
7	128.2, CH	128.1, CH	125.1, CH	120.0, CH	121.4, CH	121.4, CH	123.5, CH	123.1, CH	68.9, CH	160.2, qC	62.3, CH	62.5, CH	67.6, CH	133. 1, CH
8	157.5, qC	157.6, qC	140.6, qC	144.5, qC	136.6, qC	136.6, qC	135.2, qC	135.6, qC						
9	74.6, qC	74.6, qC	74.4, qC	74.1, qC	73.1, qC	73.2, qC	74.6, qC	74.6, qC	54.2, CH_2	63.0, CH_2	94.7, CH	94.4, CH	54.4, CH_2	54.8, CH_2
10	44.5, qC	46.2, qC	42.0, qC	40.1, qC	37.3, qC	37.3, qC	37.9, qC	37.9, qC	24.2, CH_3	19.8, CH_3	21.1, CH_3	21.1, CH_3	23.7, CH_3	58.0, CH_2
11	61.7, CH_2	61.9, CH_2	61.9, CH_2	61.7, CH_2	174.4, qC	174.4, qC	174.9, qC	174.7, qC	132.4, qC	130.6, qC	132.0, qC	31.1, CH	70.3, qC	70.3, qC
12	19.2, CH_3	19.3, CH_3	61.1, CH_2	60.6, CH_2	68.2, CH_2	68.3, CH_2	69.0, CH_2	69.0, CH_2	20.2, CH_3	19.8, CH_3	17.4, CH_3	15.7, CH_3	25.8 CH_3	25.8, CH_3
13	18.1, CH_3	18.9, CH_3	17.5, CH_3	18.3, CH_3	18.3, CH_3	18.3, CH_3	18.5, CH_3	18.5, CH_3	17.5, CH_3	16.9, CH_3	20.2, CH_3	18.7, CH_3	24.9, CH_3	24.9, CH_3
14	28.9, CH_3	33.8, CH_3	36.2, CH_3	32.6, CH_3	32.2, CH_3	32.2, CH_3	32.5, CH_3	32.5, CH_3						
15	15.5, CH_3	22.7, CH_3	23.3, CH_3	24.5, CH_3	24.3, CH_3	24.3, CH_3	24.8, CH_3	24.9, CH_3						
3a									121.8, qC	122.3, qC	122.0, qC	120.3, qC	124.5, qC	126.4, qC
5a									147.7, qC	141.4, qC	143.8, qC	145.5, qC	138.4, qC	135.5, qC
9a									124.4, qC	108.9, qC	119.0, qC	119.6, qC	121.3, qC	120.2, qC
9b									163.3, qC	160.4, qC	162.1, qC	170.9, qC	158.5, qC	158.4, qC
1′				165.7, qC	165.4, qC	165.5, qC	165.8, qC	164.6, qC						
2′				120.4, CH	119.7,CH	119.1, CH	123.0, CH	129.5, CH						
3′				144.8, CH	145.7, CH	145.8, CH	143.9, CH	141.3, CH						
4′				127.6, CH	127.8, CH	129.7, CH	130.9, CH	146.7, CH						
5′				141.4, CH	142.1, CH	142.9, CH	138.2, CH	137.5, CH						
6′				131.3, CH	131.3, CH	42.6, CH_2	101.3, CH	192.8, CH						
7′				135.3, CH	138.1, CH	65.5, CH								
8′				18.7, CH_3	42.5, CH_2	23.3, CH_3								
9′					65.7, CH									
10′					23.2, CH_3									
$-OCH_3$			53.8, CH_3				52.8 × 2, CH_3				55.7, CH_3	55.5, CH_3		

[a] Recorded in $CDCl_3$. [b] Recorded in acetone-d_6.

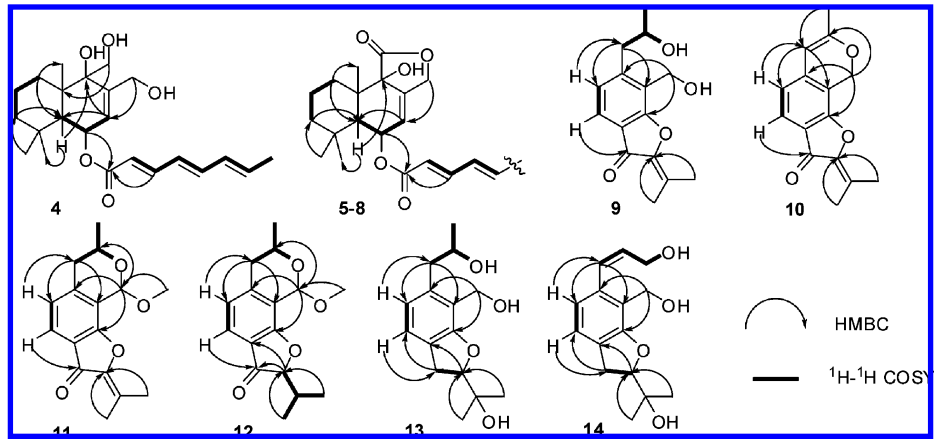


Figure 2. Key HMBC and ^{1}H−^{1}H COSY correlations for compounds **4**−**14**.

Table 3. Cytotoxicity of **4**, **6**, **8**, and **13** on the Cancer Cell Lines A549 and HL-60 (IC_{50}, μM)[a]

compound	A549	HL-60
4	30.0	20.6
6	10.5	>100
8	>100	9.0
13	>100	0.13
VP-16 (etoposide)	0.63	0.042

[a] The other new compounds (**1**−**3**, **5**, **7**, **9**−**12**, and **14**) were inactive to A549 and HL-60 cancer cell lines (IC_{50} > 100 μM).

formula of $C_{15}H_{22}O_4$, suggesting that **13** was the isomer of **15**. The 1D NMR spectra of **13** were almost identical to those of **15**,[12] except the methyl signal and methylene signal appeared as a doublet and two doublets of doublets rather than as a triplet and two multiplets, respectively. Downfield shifts for the $-CH_2-$ and $-CH_3$ and an upfield shift for the oxygenated methine were observed. The information indicated that the hydroxy group shifted to C-7 in **13** from C-6 in **15**. Thus, the structure of ustusorane E (**13**) was determined as 1-[(7-hydroxymethyl-2-(2-hydroxypropan-2-yl)-2,3-dihydrobenzofuran-6-yl)]propan-2-ol. The relative configuration at C-2 and C-7 was not determined.

The molecular formula of ustusorane F (**14**) was determined to be $C_{15}H_{20}O_4$ on the basis of HRESIMS at *m/z* 287.1258 $[M + Na]^+$. The 1D NMR data revealed that **14** also possessed the same benzofuran ring system as **13**. Detailed comparison of the 1D NMR spectra of **14** with those of **13** showed an additional disubstituted ethene moiety and an extra hydroxymethyl group at $\delta_{H/C}$ 5.78/133.1, 6.68/127.1, and 4.06/58.0, respectively. Meanwhile, the signals of the methyl, methylene, and oxygenated methine in **13** disappeared in the spectra for **14**. The extra moiety could be connected together as $-CH{=}CH-CH_2OH$ through the ^{1}H−^{1}H COSY correlations of H-6 to H-7, H-7 to H-10, and H-10 to OH-10. The HMBC correlations from H-6 to C-5 and from H-7 to C-5a confirmed the position of the $-CH{=}CH-CH_2OH$ fragment, and the *Z*-configuration of the double bond was elucidated from the value of $^3J_{H\text{-}6,\ H\text{-}7}$ (11.8 Hz). Therefore, ustusorane F (**14**) was determined as (*Z*)-3-[7-hydroxymethyl-2-(2-hydroxypropan-2-yl)-2,3-dihydrobenzofuran-6-yl]prop-2-en-1-ol.

New compounds **1**−**14** were evaluated for their cytotoxicity against A549 and HL-60 cell lines using the sulforhodamine B (SRB)[20] and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)[21] methods, respectively. Ustusorane E (**13**) exhibited strong growth inhibition against the HL-60 cell lines with an IC_{50} value of 0.13 μM. Ustusolates C (**6**) and E (**8**) exhibited moderate growth inhibition against A549 and HL-60 cell lines with IC_{50} values of 10.5 and 9.0 μM, respectively. Ustusolate A (**4**) showed weak cytotoxicity against both HL-60 and A549 cell lines with an IC_{50} value of 20.6 and 30.0 μM, respectively. The other new compounds were inactive to both human cancer cell lines (IC_{50} >100 μM).

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Beckman DU 640 spectrophotometer. CD spectra were obtained on a JASCO J-810 spectropolarimeter. IR spectra were obtained on a Nicolet NEXUS 470 spectrophotometer on KBr disks. ^{1}H NMR, ^{13}C NMR, and DEPT spectra and 2D-NMR were recorded on a JEOL JNM-ECP 600 spectrometer using TMS as internal standard, and chemical shifts were recorded as δ values. ESIMS was measured on a Q-TOF Ultima Global GAA076 LC mass spectrometer. Semipreparative HPLC was performed using an ODS column [YMC-pack ODS-A, 10 × 250 mm, 5 μm, 4 mL/min]. TLC and column chromatography (CC) were performed on plates precoated with silica gel GF_{254} (10−40 μm) and over silica gel (200−300 mesh, Qingdao Marine Chemical Factory, Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Sweden), respectively. The seawater for the culture medium of *A. ustus* was collected from the Yellow Sea near Qingdao.

Fungal Material. The 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 its morphological characteristics and analyses of ITS and 18S rRNA sequences (Supporting Information; GenBank GQ856236 and GQ856237, respectively). The voucher specimen is deposited in our laboratory at −80 °C. The working strain was prepared on potato dextrose agar slants and stored at 4 °C.

Fermentation and Extraction. The fungus *A. ustus* 094102 was grown under static conditions at 30 °C for 28 days in one hundred 1000 mL conical flasks containing liquid medium (300 mL/flask) composed of glucose (10 g/L), maltose (20 g/L), mannitol (20 g/L), monosodium glutamate (10 g/L), KH_2PO_4 (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (0.3 g/L), corn steep liquor (1 g/L), yeast extract (3 g/L), and seawater after adjusting its pH to 7.0. The fermented whole broth (30 L) was filtered through cheesecloth to separate the filtrate from the mycelia. The filtrate was concentrated under reduced pressure to about a quarter of the original volume and then extracted three times with an equivalent volume of EtOAc to give an EtOAc solution, while the mycelia were extracted three times with acetone. The acetone solution was concentrated under reduced pressure to afford an aqueous solution. The aqueous solution was extracted three times with an equivalent volume of EtOAc to give another EtOAc solution. Both EtOAc solutions were combined and concentrated under reduced pressure to give a crude gum (70.0 g).

Purification. The crude gum (70.0 g) was separated into eight fractions on a Si gel column using a step gradient elution of $CHCl_3$ and MeOH. Fr3 (10.0 g) was rechromatographyed on a Si gel column, eluted with petroleum ether/EtOAc (3:1), to provide four fractions (Fr3.1−3.4). Fr3.2 (2.7 g) was further fractionated on Sephadex LH-20 and semipreparative HPLC to give **7** (8.0 mg, t_R 7.8 min/80% MeOH) and **8** (7.8 mg, t_R 3.8 min/90% MeOH). Fr3.4 (3.1 g) was purified by repeated ODS CC and preparative HPLC to give **11** (4.0

mg, t_R 15.5 min/55% MeOH), **12** (4.5 mg, t_R 10.3 min/55% MeOH), and **10** (5.1 mg, t_R 11.9 min/80% MeOH). Fr4 (4.5 g) was chromatographyed on a Si gel column, eluted with petroleum ether/EtOAc (1:1), to provide five fractions (Fr4.1−4.5). Fr4.1 was chromatographyed over Sephadex LH-20 eluting with MeOH to obtain six subfractions (Fr4.1.1−4.1.6). Compounds **4** (20.0 mg, t_R 19.5 min) and **16** (35.5 mg, t_R 12.2 min) were obtained from Fr4.1.3 by semipreparative HPLC eluting with 80% MeOH. Fr4.2 was chromatographed over Sephadex LH-20 eluting with MeOH to obtain five subfractions (Fr4.2.1−4.2.5). Compounds **6** (3.2 mg, t_R 4.5 min) and **5** (3.0 mg, t_R 5.2 min) were obtained from Fr4.2.4 by semipreparative HPLC eluting with 85% MeOH. Fr5 (2.1 g) was further fractionationed on Sephadex LH-20 eluting with MeOH to give four subfractions (Fr5.1−5.5). Fr5.2 was purified by semipreparative HPLC to give **9** (7.8 mg, t_R 11.9 min/55% MeOH). Fr5.3 was purified by semipreparative HPLC to give **17** (11.3 mg, t_R 7.2 min/40% MeOH). Fr6 (11.1 g) was subjected to ODS CC and eluted with 30−70% aqueous MeOH to provide five fractions (Fr6.1−6.5). Fr6.3 was further chromatographed over Sephadex LH-20 eluting with MeOH to obtain seven subfractions (Fr6.3.1−6.3.7). Fr6.3.3 was purified by semipreparative HPLC to yield **13** (5.5 mg, t_R 12.5 min/50% MeOH), **14** (5.0 mg, t_R 8.6 min/55% MeOH), and **15** (6.3 mg, t_R 11.0 min/55% MeOH). Fr7 (2.2 g) was rechromatographed by ODS CC and eluted with 30−80% aqueous MeOH to provide five subfractions (Fr7.1−7.5). Fr7.2 was further purified by Sephadex LH-20 eluting with MeOH to provide five subfractions (Fr7.2.1−7.2.5). Compound **1** (12.0 mg, t_R 5.1 min/50% MeOH) was obtained from Fr7.2.4 after subjection to purification by semipreparative HPLC. Fr7.4 was rechromatographyed on a Si gel column, eluted with petroleum ether/acetone (2:1), to provide four subfractions (Fr7.4.1−7.4.4). Fr7.4.2 and Fr7.4.3 were further purified by semipreparative HPLC to give **2** (10.4 mg, t_R 6.5 min/50% MeOH) and **3** (8.5 mg, t_R 5.6 min/80% MeOH), respectively.

Cytotoxicity Bioassays. Cytotoxicity of the new compounds against A549 and HL-60 human tumor cells was determined by the SRB[20] and MTT[21] methods, respectively. Cells were plated in 96-well plates for 24 h before treatment and continuously exposed to different concentrations of compounds for 72 h. Inhibition rates of cell proliferation after compound treatments were determined by the SRB and MTT methods. VP-16 (etoposide) was used as the positive control with IC_{50} values of 0.63 and 0.042 μM on A549 and HL-60 cancer cells, respectively.

Ustusol A (1): white solid; $[\alpha]^{20}_D$ −98 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 232 (3.14) nm; CD (MeOH), λ_{max} ($\Delta\varepsilon$) 328 (+20.3), 235 (−83.2) nm; IR (KBr) ν_{max} 3520, 3440, 3380, 2920, 2835, 1660, 1635, 1435, 1380, 1240, 1195, 1100, 950, 870 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 1 and 2; HRESIMS *m/z* 269.1762 $[M + H]^+$ (calcd for $C_{15}H_{25}O_4$, 269.1753).

Ustusol B (2): colorless solid; $[\alpha]^{20}_D$ −71 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 232 (3.78) nm; CD (MeOH), λ_{max} ($\Delta\varepsilon$) 328 (+16.2), 235 (−71.5) nm; IR (KBr) ν_{max} 3400, 3320, 2950, 2928, 1658, 1615, 1460, 1385, 1290, 1208, 1155, 1078, 970 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 1 and 2; HRFABMS *m/z* 291.1577 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_4Na$, 291.1572).

Ustusol C (3): colorless solid; $[\alpha]^{20}_D$ +25 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 200 (3.26); IR (KBr) ν_{max} 3400, 2940, 2910, 1664, 1460, 1401, 1250, 1040, 960 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 1 and 2; HRESIMS *m/z* 307.1891 $[M + Na]^+$ (calcd for $C_{16}H_{28}O_4$, 307.1885).

Ustusolate A (4): colorless oil; $[\alpha]^{20}_D$ −68 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 300 (3.48) nm; IR (KBr) ν_{max} 3431, 3389, 2947, 1705, 1685, 1636, 1462, 1380, 1140, 916 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 1 and 2; HRESIMS *m/z* 391.2485 $[M + H]^+$ (calcd for $C_{23}H_{35}O_5$, 391.2484).

Ustusolate B (5): colorless oil; $[\alpha]^{20}_D$ −200 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 300 (3.56) nm; IR (KBr) ν_{max} 3400, 2958, 1778, 1705, 1663, 1449, 1380, 1100, 928 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 1 and 2; HRESIMS *m/z* 453.2265 $[M + Na]^+$ (calcd for $C_{23}H_{34}O_6Na$, 453.2253).

Ustusolate C (6): colorless oil; $[\alpha]^{20}_D$ −700 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 263 (3.44) nm; IR (KBr) ν_{max} 3395, 2926, 1770, 1703, 1660, 1470, 1392, 1006, 914 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 1 and 2; HRESIMS *m/z* 405.2293 $[M + H]^+$ (calcd for $C_{23}H_{33}O_6$, 405.2277).

Ustusolate D (7): colorless oil; $[\alpha]^{25}_D$ −300 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 255 (3.67) nm; IR (KBr) ν_{max} 3400, 2949, 1765, 1705, 1650, 1553, 1470, 1385, 1132, 917 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 1 and 2; HRESIMS *m/z* 419.2078 $[M - H]^-$ (calcd for $C_{23}H_{31}O_7$, 419.2070).

Ustusolide E (8): colorless oil; $[\alpha]^{20}_D$ −320 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 268 (3.44) nm; IR (KBr) ν_{max} 3420, 2928, 1760, 1710, 1665, 1645, 1475, 1389, 1109, 920 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 1 and 2; HRESIMS *m/z* 375.1804 $[M + H]^+$ (calcd for $C_{21}H_{27}O_6$, 375.1808).

Ustusorane A (9): pale yellow solid; $[\alpha]^{20}_D$ −28 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 347 (1.10), 282 (1.57), 276 (1.65), 209 (3.47) nm; IR (KBr) ν_{max} 3380, 1690, 1650, 1553, 1609, 1448, 1373, 1289, 1193, 1022 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 1 and 2; HRESIMS *m/z* 263.1275 $[M + H]^+$ (calcd for $C_{15}H_{19}O_4$, 263.1283).

Ustusorane B (10): yellow solid; UV (MeOH) λ_{max} (log ε) 347 (1.21), 285 (1.90), 277 (1.95), 217 (3.40) nm; IR (KBr) ν_{max} 1690, 1657, 1609, 1505, 1450, 1385, 1280, 1160, 1095 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 1 and 2; HRESIMS *m/z* 243.1019 $[M + H]^+$ (calcd for $C_{15}H_{15}O_3$, 243.1021).

Ustusorane C (11): yellow solid; $[\alpha]^{20}_D$ +6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 334 (1.20), 262 (1.85), 209 (3.51) nm; IR (KBr) ν_{max} 1695, 1648, 1605, 1510, 1448, 1389, 1290, 1117, 1002 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 1 and 2; HRESIMS *m/z* 275.1278 $[M + H]^+$ (calcd for $C_{16}H_{19}O_4$, 275.1283).

Ustusorane D (12): yellow solid; $[\alpha]^{20}_D$ −1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 328 (1.35), 258 (2.01), 213 (3.68) nm; IR (KBr) ν_{max} 1700, 1624, 1598, 1500, 1455, 1387, 1285, 1100, 1015 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 1 and 2; HRESIMS *m/z* 277.1433 $[M + H]^+$ (calcd for $C_{16}H_{21}O_4$, 277.1440).

Ustusorane E (13): colorless solid; $[\alpha]^{20}_D$ −11 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 292 (1.12), 206 (3.70) nm; IR (KBr) ν_{max} 3338, 3200, 2925, 1602, 1495, 1385, 1270, 1008 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 1 and 2; HRESIMS *m/z* 289.1422 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_4Na$, 289.1416).

Ustusorane F (14): colorless solid; $[\alpha]^{20}_D$ −2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 294 (1.32), 216 (3.76) nm; IR (KBr) ν_{max} 3257, 3300, 2910, 1648, 1616, 1505, 1369, 1287, 1019 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 1 and 2; HRESIMS *m/z* 287.1258 $[M + Na]^+$ (calcd for $C_{15}H_{20}O_4Na$, 287.1259).

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Supporting Information Available: NMR spectra of compounds **1**−**14** and the ITS and 18S rRNA sequence data of *A. ustus* 094102 are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Lu, Z. Y.; Lin, Z. J.; Wang, W. L.; Du, L.; Zhu, T. J.; Fang, Y. C.; Gu, Q. Q.; Zhu, W. M. *J. Nat. Prod.* **2008**, *71*, 543–546.
(2) Wang, W. L.; Lu, Z. Y.; Tao, H. W.; Zhu, T. J.; Fang, Y. C.; Gu, Q. Q.; Zhu, W. M. *J. Nat. Prod.* **2007**, *70*, 1558–1564.
(3) Du, L.; Zhu, T. J.; Fang, Y. C.; Gu, Q. Q.; Zhu, W. M. *J. Nat. Prod.* **2008**, *71*, 1343–1351.
(4) Horak, R. M.; Steyn, P. S.; Vleggaar, R.; Rabie, C. J. *J. Chem. Soc., Perkin Trans. 1* **1985**, *2*, 345–356.
(5) Steyn, P. S.; Vleggaar, R. *J. Chem. Soc., Perkin Trans. 1* **1974**, *19*, 2250–2256.
(6) Horak, R. M.; Steyn, P. S.; Vleggaar, R.; Rabie, C. J. *J. Chem. Soc., Perkin Trans. 1* **1985**, *2*, 363–367.
(7) Cutler, H. G.; Crumley, F. G.; Springer, J. P.; Cox, R. H. *J. Agric. Food Chem.* **1981**, *29*, 981–983.
(8) Kohno, J.; Hiramatsu, H.; Nishio, M.; Sakurai, M.; Okuda, T.; Komatsubara, S. *Tetrahedron* **1999**, *55*, 11247–11252.
(9) Kohno, J.; Sakurai, M.; Kameda, N.; Nishio, M.; Kawano, K.; Kishi, N.; Okuda, T.; Komatsubara, S. *J. Antibiot.* **1999**, *52*, 913–916.
(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.; Charlton, P.; Doherty, A. M. *J. Antibiot.* **1996**, *49*, 505–512.
(11) Singh, S. B.; Simith, J. L.; Sabnis, G. S.; Dombrowski, A. W.; Schaeffer, J. M.; Goetz, M. A.; Bills, G. F. *Tetrahedron* **1991**, *47*, 6931–6938.

(12) Shao, H. J.; Qin, X. D.; Dong, Z. J.; Zhang, H. B.; Liu, J. K. *J. Antibiot.* **2008**, *61*, 115–119.
(13) Cutler, H. G.; Crumley, F. G.; Springer, J. P.; Cox, R. H.; Cole, R. J.; Dorner, J. W.; Thean, J. E. *J. Agric. Food Chem.* **1980**, *28*, 989–991.
(14) Uosaki, Y.; Yoshida, M.; Ogawa, T.; Saitoh, Y. *J. Antibiot.* **1996**, *49*, 6–12.
(15) Ye, X. L. *Stereochemistry*; Beijing University Express: Beijing, 1999; pp 257−259.
(16) Garlaschelli, L.; Vidari, G. *Tetrahedron* **1989**, *45*, 7371–7378.
(17) Grabley, S.; Thiericke, R.; Zerlin, M. *J. Antibiot.* **1996**, *49*, 593−595.
(18) Ogawa, A.; Murakami, C.; Kamisuki, S.; Kuriyama, I.; Yoshida, H.; Sugawara, F.; Mizushina, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3539–3543.
(19) Saito, F.; Kuramochi, K.; Nakazaki, A.; Mizushina, Y.; Sugawara, F.; Kobayashi, S. *Eur. J. Org. Chem.* **2006**, 4796–4799.
(20) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.
(21) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.

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