Polyketide and Sesquiterpenediol Metabolites from a Marine-Derived Fungus

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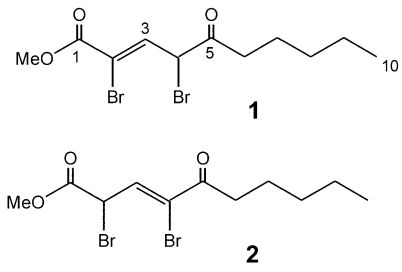
Marine microorganisms such as bacteria and fungi inhabit virtually any environment in the sea, and they are rich sources of chemically and biologically diverse compounds.[1,2]

In our search for bioactive compounds in marine microorganisms,[3] two new halogenated alkenoates, methyl 2,4-dibromo-5-oxo-2-decenoate (**1**) and methyl 2,4-dibromo-5-oxo-3-decenoate (**2**), and the known sesquiterpenediol, cyclonerodiol (**3**), were isolated from the broth of an unidentified fungus, which was separated from the surface of the marine red alga *Gracillaria verrucosa* collected at Jinha, Ulsan in 2002.



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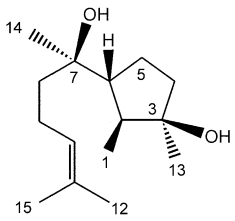

cyclonerodiol (**3**)

The fungus was cultured (10 L) in a seawater-based medium.[4] The resulting broth and mycelium were extracted separately to afford crude extracts of 0.7 g and 6.5 g, respectively. The broth extract (EtOAc) was subjected to a combination of column chromatography on silica gel (*n*-hexane/EtOAc) and octadesyl silica (ODS) gel (H_2O/MeOH) to furnish the fractions containing compounds **1** and **2** (20 mg), and compound **3** (25 mg). Further purification of each fraction by HPLC (YMC ODS-A, MeOH-H_2O = 5 : 1) yielded compounds **1** (5.5 mg), **2** (8.0 mg), and **3** (11 mg), respectively.

Compound **2**[5] was isolated as a yellow oil which was thought to have a molecular composition of $C_{11}H_{16}Br_2O_3$ from the high resolution (HR) FABMS and ^{13}C NMR data.

Three degree of unsaturation in HRFABMS implied that **2** contained two carbonyls and one double bond. The quasi-molecular ions were observed at m/z 355, 357, and 359 with the ratio 1 : 2 : 1, indicating that compound **2** has two bromine atoms. The IR spectrum of **2** showed absorptions for ester (1743, 1137 cm^{-1}) and enone (1697, 1265 cm^{-1}) functionality. The UV spectrum also exhibited the presence of an enone chromophore [257 nm (log ε 3.5)].

In the ^1H NMR spectrum, the presence of an ester methyl proton [δ 3.85 (3H, s, 1-OCH_3)], an olefinic proton [δ 7.43 (1H, d, J = 9.8 Hz, H-3)], an allyl proton [5.28 (1H, d, J = 9.8 Hz, H-2)], and *n*-pentyl protons was inferred. Detailed analyses of the ^1H and ^{13}C NMR spectra of **2**, including the results from COSY, DEPT, HMQC, and HMBC experiments, revealed signals ascribable to a methyl ester [δ 3.85 (3H, s, 1-OCH_3), 167.5 (C-1), 53.7 (1-OMe)], 1,2,4,4-tetrasubstituted-2-buten-1-one [δ 5.28 (1H, d, J = 9.8 Hz, H-2), 7.43 (1H, d, J = 9.8 Hz, H-3)], 40.9 (C-2), 135.4 (C-3), 130.4 (C-4), 193.7 (C-5)], and n-pentyl moiety [δ 2.82 (2H, t, J = 7.3 Hz, H_2-6), 1.66 (2H, m, H_2-7), 1.32 (4H, m, H_2-8/9), 0.91 (3H, t, J = 6.6 Hz, H_3-10), 38.8 (C-6), 23.9 (C-7), 31.2 (C-8), 22.4 (C-9), 13.9 (C-10)] (Table 1).

The connection of the functional groups in **2**, which led to the planar structure, was achieved on the basis of HMQC and HMBC data. Key HMBC correlations from 1-OCH_3 to C-1, from H-2 to C-1, C-3, and C-4, from H-3 to C-1 and C-5, from H-6 to C-5, and from H-7 to C-5 were critical in establishing the planar structure of **2**.

Two bromines were confirmed to attach to C-2 and C-4 by the HMBC correlations between H-2 and C-1, C-3, and C-4, as well as the characteristic mass fragments of *m/z* 99 $[C_5H_{11}CO]^+$ and 203 $[M\text{-}CH_3OCOCHBr]^+$.

Compound **1**[6] was obtained as a yellow oil, and HRFABMS

Table 1. ^1H (δ, mult, J) and ^{13}C (δ, mult) NMR Data for Methyl 2,4-Dibromo-5-oxo-2-decenoate (**1**) and Its 3-decenoate (**2**)[a]

Carbon No.	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1		161.9 (s)		167.5 (s)
2		120.5 (s)	5.28 (d, 9.8)	40.9 (d)
3	7.63 (d, 9.9)	137.7 (d)	7.43 (d, 9.8)	135.4 (d)
4	5.22 (d, 9.9)	47.4 (d)		130.4 (s)
5		199.9 (s)		193.7 (s)
6	2.85 (dt, 17.3, 7.3)	39.8 (t)	2.82 (t, 7.3)	38.8 (t)
	2.61 (dt, 17.3, 7.3)			
7	1.66 (m)	23.5 (t)	1.66 (m)	23.9 (t)
8	1.32 (m)	31.1 (t)	1.32 (m)	31.2 (t)
9	1.32 (m)	22.3 (t)	1.32 (m)	22.4 (t)
10	0.90 (t, 6.9)	13.9 (q)	0.91 (t, 6.6)	13.9 (q)
1-OMe	3.87 (s)	53.8 (q)	3.85 (s)	53.7 (q)

[a]Recorded in $CDCl_3$ at 400 MHz (^1H) and 100 MHz (^{13}C).

and ^{13}C NMR methods established the molecular formula to be $C_{11}H_{16}Br_2O_3$. The general features of its UV. IR and NMR spectra (Table 1) closely resembled those of compound **2**. except that the coupling pattern of NMR signal assigned to the methylene (H_2-6) was changed from triplet [δ 2.82 (2H. t, J = 7.3 Hz. H_2-6)] for **2** to doublet of triplet [δ 2.61 (1H, dt. J = 17.3, 7.3 Hz, H_a-6) and 2.85 (1H. dt. J = 17.3. 7.3 Hz. H_b-6)] for **1** (Table 1).

Detailed analyses of the ^1H and ^{13}C NMR spectra of **1**. including the results from DEPT. COSY. HMQC. and HMBC experiments. suggested that the metabolite **1** is the positional isomer of double bond of compound **2**.

The location of double bond of the metabolite **1** was determined by the HMBC data, in which diagnostic correlations from H-4 to C-2. C-3. and C-5. and from H-6 to C-5, C-7. and C-8 showed the C2-C3 double bond in **1**.

On the basis of all of the foregoing evidence, the structures of compounds **1** and **2** were determined as methyl 2.4-dibromo-5-oxo-2-decenoate and methyl 2.4-dibromo-5-oxo-3-decenoate. respectively.

Cycloneredіol (**3**).[7] a sesquiterpenediol, was first reported as a metabolite of the fungus *Trichothecium reseum*.[8,9] Subsequent isolations were made from *Gibberella fujikuroi*,[10] *Fusarium culmorum*,[11] and *Trichoderma koningii* as the plant growth regulatory active constituent.[12] The biosynthetic pathway has been specifically established with the cell-free extracts of *G. fujikuroi*[13] for cyclonerodiol.

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References and Notes

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4. The fungus was cultured for 30 days (static) at 29 °C in SWS medium: soytone (0.1%), soluble starch (1.0%), and seawater (100%).
5. Methyl 2.4-dibromo-5-oxo-3-decenoate (**2**) was isolated as a yellow oil which showed: $[\alpha]_D$ -40 °C (*c* 0.2. $CHCl_3$); IR (KBr): 2952. 2930. 2856. 1743. 1697. 1437. 1265. 1137 cm^{-1}; UV (MeOH): 203 (logε 3.7). 257 (3.5) nm; LREIMS *m/z* 359 $[M+H]^+$ (0.4), 357 $[M+H]^+$ (0.9), 355 $[M+H]^+$ (0.4), 333 (0.6). 331 (1.1). 329 (0.7), 302 $[M-H-C_4H_9]^-$ (3). 300 $[M+H-C_4H_9]^+$ (6). 298 $[M+H-C_4H_9]^+$ (3). 277 $[M-Br]^+$ (8). 275 $[M-Br]^+$ (8). 259 $[M-C_4H_9-CH_2CO]^-$ (1.9). 257 $[M-C_4H_9-CH_2CO]^+$ (3.3). 255 $[M-C_4H_9-CH_2CO]^-$ (1.7). 245 (14). 243 (15). 221 (22). 219 (21). 203 (12). 189 (26), 187 (27), 149 (49), 99 (100). 71(76); LRFABMS *m/z* 355 $[M-H]^-$. 357 $[M+H]^+$. 359 $[M+H]^+$; HRFABMS *m/z* 354.9544 (calcd for $C_{11}H_{17}O_3\ ^{79}Br_2$, 354.9545), 356.9521 (calcd for $C_{11}H_{17}O_3\ ^{79}Br^{81}Br$. 356.9524). 358.9506 (calcd for $C_{11}H_{17}O_3\ ^{81}Br_2$. 358.9504); See Table 1 for NMR spectral data.
6. Methyl 2.4-dibromo-5-oxo-2-decenoate (**1**) was isolated as a yellow oil which showed: $[\alpha]_D$ -10 °C (*c* 0.2, $CHCl_3$); IR (KBr): 2952, 2930, 2856. 1734, 1436. 1240, 1040. 751 cm^{-1}; UV (MeOH): 203 (logε 3.8), 248 (3.3) nm; LRFABMS *m/z* 355 $[M+H]^-$. 357 $[M+H]^+$. 359 $[M+H]^+$; HRFABMS *m/z* 354.9543 (calcd for $C_{11}H_{17}O_3\ ^{79}Br_2$. 354.9544). 356.9522 (calcd for $C_{11}H_{17}O_3\ ^{79}Br^{81}Br$. 356.9524). 358.9506 (calcd for $C_{11}H_{17}O_3\ ^{81}Br_2$. 358.9506); See Table 1 for NMR spectral data.
7. Cyclonerodiol (**3**) was isolated as a yellow oil which showed spectral data virtually identical to those reported in the literature[12] except for the assignment of NMR data. The NMR data were reassigned as follow: ^1H-NMR ($CDCl_3$) δ: 1.05 (3H. d. J = 7.0 Hz. H_3-1). 1.59 (1H. m. H-2). 1.57. 1.59 (each 1H. m. H_2-4). 1.86 (2H. m. H_2-5), 1.83 (1H. m. H-6), 1.49 (2H. t, J = 8.3 Hz. H_2-8), 2.05 (2H. m. H_2-9), 5.12 (1H, t. J = 7.0 Hz. H-10). 1.69 (3H, s, H_3-12). 1.26 (3H, s, H_3-13), 1.17 (3H. s. H_3-14). 1.63 (3H. s, H_3-15). ^{13}C-NMR ($CDCl_3$) δ: 14.5 (C-1). 44.2 (C-2). 81.3 (C-3). 40.4 (C-4). 24.3 (C-5). 54.2 (C-6). 74.8 (C-7). 40.4 (C-8). 22.6 (C-9). 124.5 (C-10). 131.7 (C-11). 25.7 (C-12). 26.1 (C-13). 25.0 (C-14). 17.7 (C-15).
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