Polyketide and Sesquiterpenediol Metabolites from a Marine-Derived Fungus

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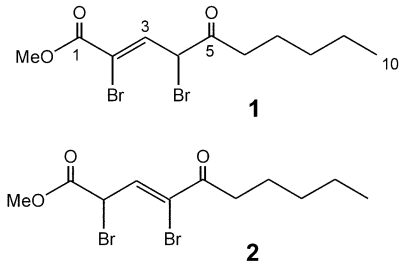
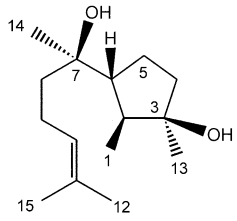
Received March 2, 2004

Key Words : Marine-derived fungus, Brominated alkenoate, Methyl 2,4-dibromo-5-oxo-2-decenoate, Methyl 2,4-dibromo-5-oxo-3-decenoate, Cyclonerodiol

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, cycloneroidol (**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.





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) 2.61 (dt, 17.3, 7.3)	39.8 (t)	2.82 (t, 7.3)	38.8 (t)
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.

Cyclonerediol (**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.

Acknowledgements. Mass spectral data was kindly provided by the Korea Basic Science Institute. This work was supported by the Brain Korea 21 Project in 2003 (F020).

References and Notes

1. Faulkner, D. J. *Nat. Prod. Rep.* **2002**, *19*, 1-47.
2. Pietra, F. *Nat. Prod. Rep.* **1997**, *14*, 453-464.
3. Li, X.; Lee, S. M.; Choi, H. D.; Kang, J. S.; Son, B. W. *Chem. Pharm. Bull*. **2003**, *51*, 1458-1459.
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).
8. Nozoe, S.; Goi, M.; Morisaki, N. *Tetrahedron Lett*. **1970**, *15*, 1293.
9. Evans, R.; Hanson, J. R.; Nyfeler, R. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1214.
10. Cross, B. E.; Markwell, R. E.; Stewart, J. C. *Tetrahedron* **1971**, *27*, 1663.
11. Hanson, J. R.; Hitchcock, P. B.; Nyfeler, R. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1586.
12. Cutler, H. G.; Jacyno, J. M.; Phillips, R. S.; VonTersch, R. L.; Cole, P. D.; Montemurro, N. *Agric. Biol. Chem*. **1991**, *55*, 243.
13. Cane, D. E.; Iyengar, R.; Shiao, M.-S. *J. Am. Chem. Soc*. **1981**, *103*, 914.