Induced Production of Bromomethylchlamydosporols A and B from the Marine-Derived Fungus *Fusarium tricinctum*

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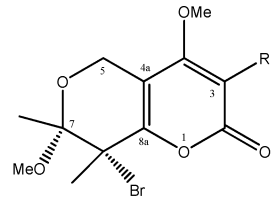
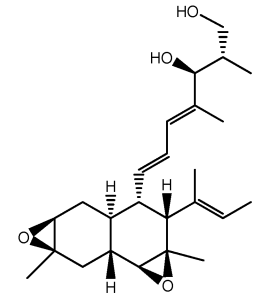
The addition of $CaBr_2$ to the fermentation of a marine-derived *Fusarium tricinctum* resulted in production of halogenated chlamydosporol analogues. Two new antimicrobial halogenated pyranopyranones, bromomethylchlamydosporols A (**1**) and B (**2**), and two known compounds, chlamydosporol (an inseparable epimeric mixture of 7*R*:7*S* = 1:1 from ^{1}H NMR data) (**3**) and fusarielin A (**4**), were isolated from the culture. The structures of **1** and **2** were assigned through a combination of spectroscopic data analyses. Compounds **1**−**4** exhibited mild antibacterial activity against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, and multidrug-resistant *S. aureus*. The MIC values of each strain were as follows: compounds **1** and **2** showed an MIC of 15.6 μg/mL against *S. aureus*, methicillin-resistant *S. aureus*, and multidrug-resistant *S. aureus*, and compounds **3** and **4** exhibited an MIC of 31.5 μg/mL against *S. aureus* and methicillin-resistant *S. aureus* and 62.5 μg/mL against multidrug-resistant *S. aureus*.

Marine-derived microorganisms continue to attract attention as a rich source of structurally novel bioactive metabolites that are potential lead compounds for the development of new drugs.[1,2] When cultured under saline condition, these microorganisms produced biologically active halogenated metabolites, for example salinosporamide A,[3] a highly potent inhibitor of the 20S proteasome, and its halogenated derivatives,[4] cytotoxic halogenated polyenyl pyrroles, isorumbrin and bromoisorumbrin,[5] nematicidal and antimicrobial lachnumon and mycorrhizin A derivatives,[6] bromomyrothenone B,[7] and the antibacterial chlorohydroaspyrones A and B.[8] Encouraged by the detection of halogenated marine analogues, we added halide salts to cultures of marine-derived microorganisms in an effort to gain access to additional halogenated secondary metabolites, and using this method we produced the antioxidant bromochlorogentisylquinones A and B.[9] A crude extract from a small-scale culture of *Fusarium tricinctum* displayed antimicrobial activity against *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), and multidrug-resistant *S. aureus*. A larger culture (20 L) was then grown to facilitate the purification of bioactive metabolites and to search for new bioactive metabolites by adding halide salts to the culture medium. This report describes the production, isolation, identification, and antibacterial activity of two new antimicrobial halogenated pyranopyranones (**1**, **2**) and two known compounds, chlamydosporol (7*R*:7*S* = 1:1 from ^{1}H NMR data) (**3**)[10] and fusarielin A (**4**).[11]



Bromomethylchlamydosporol
A (**1**): R = H
B (**2**): R = Br

Epimers of chlamydosporol (**3**)
(7*R*:7*S* = 1:1)



Fusarielin A (**4**)

Bromomethylchlamydosporol A (**1**) was obtained in the form of a yellow solid. It showed an isotopic cluster at *m*/*z* 318 [M (^{79}Br)]$^+$ and 320 [M (^{81}Br)]$^+$ with a 1:1 ratio in the EIMS, suggesting the presence of one bromine atom. A molecular formula of $C_{12}H_{15}BrO_5$, which gave five degrees of unsaturation, was established by HREIMS and ^{13}C NMR methods. Detailed analyses of the ^{1}H and ^{13}C NMR spectra of **1**, including DEPT, COSY, HMQC, and HMBC experiments, revealed signals that were assigned to two quaternary methyls, two methoxyls, one oxymethylene, and a 4,5,6-trisubstituted α-pyrone (Table 1). The α-pyrone was further supported by IR (1729 and 1574 cm^{-1}) and UV [296 nm (log ε = 6.7)] spectral data. The connectivity of functional groups in **1** was elucidated on the basis of HMBC techniques. The key HMBC correlations, from H-3 to C-2, C-4, and C-4a, from 4-OCH_3 to C-4, from H_2-5 to C-4, C-4a, C-7, and C-8a, from 7-CH_3 to C-7 and C-8, from 7-OCH_3 to C-7, and from 8-CH_3 to C-7, C-8, and C-8a, showed the connections among C4a−C5, C5−O−C7, and C7−C8−C8a as well as the positions of the 8-bromo, 4,7-dimethoxy, and 7,8-dimethyl groups. These spectroscopic features revealed that compound **1** had the general structural features of chlamydosporol (**3**).[10] The NMR data for both compounds showed similar patterns, except that a hydroxyl proton [δ_H 6.24 (1H, s, 7-OH)] in chlamydosporol was replaced with a methoxyl group [δ_H 3.31 (3H, s, 7-OCH_3), δ_C 57.2 (CH_3, 7-OCH_3)] in **1**, and an sp^3-methine [δ_H 2.72 (1H, q, *J* = 7.5 Hz, H-8), δ_C 39.7 (CH, C-8)] in chlamydosporol was replaced with an sp^3-quaternary carbon [δ_C 61.6 (qC, C-8)] in **1**. Thus, compound **1** was characterized as the 8-bromo-7-*O*-methyl derivative of chlamydosporol, and a direct comparison of NMR data for **1** with those for chlamydosporol provided additional support for the planar structure shown for **1**. The relative configurations at the asymmetric centers C-7 and C-8 of **1** were assigned by NOESY experiments (see Figure S1 in the Supporting Information). The NOESY correlations between 7-CH_3

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Table 1. NMR Spectroscopic Data for Bromomethylchlamydosporols A (**1**) and B (**2**)[a]

	bromomethylchlamydosporol A (**1**)		bromomethylchlamydosporol B (**2**)	
position	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H, (J in Hz)
2	161.9, qC		159.1, qC	
3	89.6, CH	5.73. s	94.5, qC	
4	167.6, qC		165.3, qC	
4a	104.9, qC		107.9, qC	
5	56.5, CH_2	4.24, d (15.6)	56.7, CH_2	4.32, d (16.1)
		4.48, d (15.6)		4.59, d (16.1)
7	101.8, qC		101.7, qC	
8	61.6, qC		61.2, qC	
8a	154.5, qC		153.4, qC	
7-Me	18.5, CH_3	1.67, s	18.4, CH_3	1.69, s
8-Me	23.3, CH_3	1.88, s	23.3, CH_3	1.90, s
4-OMe	51.3, CH_3	3.83, s	51.3, CH_3	4.12, s
7-OMe	57.2, CH_3	3.31, s	61.6, CH_3	3.33, s

[a] Recorded in DMSO-d_6 at 400 MHz (^{1}H) and 100 MHz (^{13}C).

and 8-CH_3 and between H_a-5 [δ_H 4.24 (1H, d, J = 15.6 Hz)] and 7-OCH_3 suggested that both groups were on the same face. Molecular modeling based on the NOESY data suggested that 7-OCH_3 was oriented α-axially [see Figure S1(a)] or β-axially [see Figure S1(b)]. Therefore, the 7- and 8-methyl groups were oriented β-equatorially and β-pseudoaxially [see Figure S1(a)] or α-equatorially and α-pseudoaxially [see Figure S1(b)], respectively. The absence of a NOESY correlation between 7-OCH_3 and 8-CH_3 favored the β-pseudoaxial [see Figure S1(a)] or α-pseudoaxial [see Figure S1(b)] orientation for 8-CH_3 rather than the alternative α-equatorial or β-equatorial configuration. Therefore, the bulky bromo group was equatorial (see Figure S1 in the Supporting Information). Thus, the relative configurations at C-7 and C-8 in **1** were presumed to be 7*R** and 8*S**, as shown.

Bromomethylchlamydosporol B (**2**) was obtained in the form of a yellow solid. Compound **2** showed isotopic clusters at *m*/*z* 396 [M ($^{79}Br_2$)]$^+$ (6), 398 [M ($^{79}Br^{81}Br$)]$^+$ (11), and 400 [M ($^{81}Br_2$)]$^+$ (5) with a ratio of 1:2:1 in the EIMS, suggesting the presence of two bromine atoms. A molecular formula of $C_{12}H_{14}Br_2O_5$, which gave five degrees of unsaturation, was established by HREIMS and ^{13}C NMR methods. The general features of the UV, IR, and NMR spectra closely resembled those of bromomethylchlamydosporol A (**1**), except that an sp^2-methine [δ_H 5.73 (1H, s, H-3), δ_C 89.6 (CH, C-3)] in **1** was substituted with an sp^2-quaternary carbon [δ_C 94.5 (qC, C-3)] in **2** (Table 1). Detailed analyses of the ^{1}H and ^{13}C NMR spectra of **2**, including the results from DEPT, COSY, HMQC, and HMBC experiments, suggested that **2** was a 3-bromide derivative of **1**, 3,8-dibromo-7-*O*-methylchlamydosporol. Thus, the planar structure of bromomethylchlamydosporol B was confidently assigned. As in **1**, the relative stereochemistry of **2** was deduced by comparison of the chemical shifts of protons and carbons in the 1,2-dihydro-5*H*-pyran moiety with those of **1**. This was further supported by the NOE correlations between 7-CH_3 and 8-CH_3 and between H_a-5 [δ_H 4.32 (1H, d, J = 16.1 Hz)] and 7-OCH_3. On the basis of the above evidence, the relative configurations of bromomethylchlamydosporol B (**2**) and bromomethylchlamydosporol A (**1**) were identical.

The known inseparable epimeric mixture of chlamydosporol (7*R*:7*S* = 1:1 from ^{1}H NMR data) and fusarielin A were also obtained in this investigation. They were identified by inspecting their NMR spectra and comparing these data with literature values.[10,11]

Compounds **1**−**4** exhibited a mild antibacterial activity against *S. aureus*, MRSA, and multidrug-resistant *S. aureus*. The MICs of each strain were as follows: compounds **1** and **2** showed an MIC of 15.6 μg/mL for *S. aureus*, MRSA, and multidrug-resistant *S. aureus*, and compounds **3** and **4** exhibited an MIC of 31.5 μg/mL for *S. aureus* and MRSA and 62.5 μg/mL for multidrug-resistant *S. aureus*.

Experimental Section

General Experimental Procedures. Optical rotation was determined on a Perkin-Elmer model 341 polarimeter. UV/visible spectra were measured on a Hitachi U-2001 UV/vis spectrometer. IR spectra were recorded on a Bruker FT-IR model IFS-88 spectrometer. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained on a JEOL JNM-ECP 400 NMR spectrometer, using TMS or solvent peaks [DMSO-d_6: ^{1}H (δ 2.50) and ^{13}C (δ 39.5)] as reference standard. MS spectra were obtained on a JEOL JMS-700 spectrometer. HPLC was performed on a Young LIN-ACME HPLC system using a reversed-phase analytical column (Gemini C18, 4.6 × 250 mm, 5 μm) with UV detection.

Fungal Isolation and Culture. The fungal strain, *Fusarium tricinctum*, was isolated from the edible marine brown alga *Sargassum ringgoldium* (Korean name: KeunIp MoJaBan) collected in GeoMun Island, Yeosu, Korea, and identified on the basis of 18S rRNA analyses (SolGent Co., Ltd., Daejeon, Korea), identity of 99%. A voucher specimen is deposited at Pukyong National University with the code MFB392-2. The fungus was cultured (1 L × 10) in SWS medium consisting of soytone (0.1%), soluble starch (1.0%), and seawater (100%). The cultures were incubated at 29 °C for 10 days on a rotary shaker (120 rpm), and $CaBr_2$ (50 mM)[6,12] was subsequently added. Incubation was further continued for 10 days under the same conditions. The culture control was incubated in the absence of $CaBr_2$ in the same manner as described above. TLC analysis showed that the composition of the extract differed from the extract derived from bromide-free SWS medium.

Extraction and Isolation. The mycelium and broth were separated by filtration using cheeesecloth. The filtered broth was extracted with EtOAc to afford broth extract (650 mg), which was subjected to Si gel flash chromatography. Elution was performed with *n*-hexane−EtOAc (stepwise, 0−100% EtOAc) to yield four fractions. Fraction 2, which exhibited antimicrobial activity against *S. aureus*, MRSA, and multidrug-resistant *S. aureus*, was separated by medium-pressure liquid chromatography (MPLC) (ODS) using a H_2O−MeOH gradient elution to afford crude compounds **1** and **2**. These were further purified by HPLC (Gemini C18, 4.6 × 250 mm, 5 μm, 1 mL/min) utilizing a 30 min gradient program of 50% to 100% MeOH in H_2O to furnish **1** (5.5 mg) and **2** (3.0 mg). Chlamydosporol (32 mg), an inseparable epimeric mixture (7*R*:7*S* = 1:1 from ^{1}H NMR data), and fusarielin A (11.0 mg) were isolated from fraction 3, which also showed antimicrobial activity, by the same chromatographic method above.

Bromomethylchlamydosporol A (1): yellow solid; $[\alpha]_D^{20}$ +2.2 (*c* 0.5, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 214 (6.9), 296 (6.7); IR (neat) ν_{max} 2970, 1729, 1574, 1460, 1373, 1253, 1103, 1060 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 320 [M, ^{81}Br]$^+$ (7.6), 318 [M, ^{79}Br]$^+$ (7.0), 246 (66), 244 (60), 208 (50), 207 (61), 197 (100), 165 (51), 137 (43), 109 (25), 69 (57), 52 (34); HREIMS *m*/*z* 318.0100 [M(^{79}Br)]$^+$ (calcd for $C_{12}H_{15}{}^{79}BrO_5$, 318.0103) (Δ −0.8 ppm), *m*/*z* 320.0081 [M]$^+$ (calcd for $C_{12}H_{15}{}^{81}BrO_5$, 320.0084) (Δ −0.3 ppm).

Bromomethylchlamydosporol B (2): yellow solid; $[\alpha]_D^{20}$ +1.8 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 315 (4.0); IR (neat) ν_{max} 2985, 1737, 1374, 1245, 1047, 757 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 400 [M ($^{81}Br_2$)]$^+$ (5), 398 [M (^{81}Br, ^{79}Br)]$^+$ (11), 396 [M ($^{79}Br_2$)]$^+$ (6), 326 (18), 324 (41), 322 (22), 296 (28), 288 (30), 287 (38), 277 (98), 275 (100), 243 (16), 215(37), 187 (43), 147 (24), 93

(32), 83 (64), 65 (52), 53 (63); HREIMS *m/z* 395.9211 $[M(^{79}Br_2)]^+$ (calcd for $C_{12}H_{14}{}^{79}Br_2O_5$, 395.9208) ($\Delta$ +0.7 ppm), *m/z* 397.9198 $[M(^{79}Br, {}^{81}Br)]^+$ (calcd for $C_{12}H_{14}{}^{79}Br{}^{81}BrO_5$, 397.9188) ($\Delta$ +2.7 ppm), *m/z* 399.9167 $[M]^+$ (calcd for $C_{12}H_{14}{}^{81}Br_2O_5$, 399.9170) ($\Delta$ −0.1 ppm).

Inseparable Epimeric Mixture of Chlamydosporol (3) and Fusarielin A (4). Spectroscopic data were virtually identical to those reported in the literature.[10,11]

Antibacterial Assay. [13] The *in vitro* antibacterial activity of the fermentation broth and purified samples was evaluated by a conventional 2-fold serial dilution method using *S. aureus*, MRSA, and multidrug-resistant *S. aureus* as indicator strains. These strains (*S. aureus* ATCC29213, methicillin-resistant *S. aureus* CCARM3167, and multidrug-resistant *S. aureus* CCARM3089) were obtained from Culture Collection of Antimicrobial Resistant Microbes of Korea (CCARM). A 5 mL suspension containing 10^5 cells per mL was used as inoculum of the test organism. The MICs were determined after inoculation for 18 h at 37 °C.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **1** and **2** (in DMSO-d_6) and proposed stereochemistry of **1** including key NOE correlations. These materials are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2010**, *27*, 165–237.
(2) Jensen, P. R.; Fenical, W. In *Marine Microorganisms and Drug Discovery: Current Status and Future Potential. Drugs from the Sea* Fusetani, N., Ed.; Karger: Basel, 2000; pp 6−29.
(3) Feling, R. H.; Buchanan, G. O.; Mincer, T. J.; Kaufman, C. A.; Jensen, P. R.; Fenical, W. *Angew. Chem., Int. Ed.* **2003**, *42*, 355–357.
(4) Lam, K. S.; Tsueng, G.; McArthur, K. A.; Mitchell, S. S.; Potts, B. C. M.; Xu, J. *J. Antibiot.* **2007**, *60*, 13–19.
(5) Clark, B. R.; Lacey, E.; Gill, J. H.; Capon, R. J. *J. Nat. Prod.* **2007**, *70*, 665–667.
(6) Stadler, M.; Anke, H.; Sterner, O. *J. Antibiot.* **1995**, *48*, 149–153.
(7) Li, X.; Zhang, D.; Lee, U.; Li, X.; Cheng, J.; Zhu, W.; Jung, J. H.; Choi, H. D.; Son, B. W. *J. Nat. Prod.* **2007**, *70*, 307–309.
(8) Zhang, D.; Yang, X.; Kang, J. S.; Choi, H. D.; Son, B. W. *J. Nat. Prod.* **2008**, *71*, 1458–1460.
(9) Nenkep, V. N.; Yun, K.; Li, Y.; Choi, H. D.; Kang, J. S.; Son, B. W. *J. Antibiot.* **2010**, *63*, 199–201.
(10) (a) Grove, J. F.; Hitchcock, P. B. *J. Chem. Soc., Perkin Trans. 1* **1991**, 997–999. (b) Solfrizzo, M.; Forbes-Smith, M.; Strange, R. N.; Visconti, A. *Chromatographia* **1994**, *39*, 443–447. (c) Visconti, A.; Solfrizzo, M.; Fruchier, A.; ApSimon, J. W. *J. Nat. Prod.* **1994**, *57*, 695–699.
(11) Nguyen, H. P.; Zhang, D.; Lee, U.; Kang, J. S.; Choi, H. D.; Son, B. W. *J. Nat. Prod.* **2007**, *70*, 1188–1190.
(12) Stadler, M.; Anke, H.; Sterner, O. *J. Antibiot.* **1995**, *48*, 261–266.
(13) Li, Y.; Li, X.; Son, B. W.; Choi, H. D. *Kor. J. Pharmacogn.* **2003**, *34*, 142–144.

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