Original Article

Natural Product Communications
Volume 15(3): 1–4

DOI: 10.1177/1934578X20902886
journals.sagepub.com/home/npx

SAGE

A New Chlorinated Phenolic Compound From the Antarctic Lichen, *Pertusaria dactylina*

Man H. Koo[1], Min J. Kim[1], Jae E. Seo[2], Ji H. Kim[2], Se J. Han[2,3], Il C. Kim[2], Jun H. Lee[1,2], and Ui J. Youn[2,3]

Abstract

A new chlorinated phenolic compound, methyl-3-chloro-2-hydroxy-4-methoxy-6-pentylbenzoate (**1**) and 4 known compounds (**2**-**5**) were isolated from the Antarctic lichen, *Pertusaria dactylina* (*Pertusariaceae*). The structure of the new compound was determined by means of One-dimensional and two dimensional nuclear magnetic resonance (1D and 2D NMR) and high-resolution fast atom bombardment mass spectrometry (HRFABMS) experiments. The antimicrobial activities of compounds **1** to **5** against *Staphylococcus aureus* and *Candida albicans* were evaluated. The results showed that compound **1** exhibited a weak inhibitory effect against *C. albicans* with an IC_{50} value of 67 ± 7 μg/mL.

Keywords

Pertusaria dactylina, Antarctic lichen, *Pertusariaceae*, antimicrobial activity, phenolic compound

Received: October 3rd, 2019; Accepted: December 26th, 2019.

Lichens are symbiotic systems consisting of a mycobiont (the dominant fungal partner), one or more photobionts (algal partner), and a complex microbial consortium comprising a wide array of heterotrophic bacteria and fungi.[1,2] More than 1000 metabolites have so far been described from lichen sources, displaying various biological activities, such as antibiotic, antimycobacterial, antioxidant, antitumor, antiviral, analgesic, and antipyretic properties.[3] The *Pertusaria* genus, which contains about 800 species, is globally distributed from the tropics to the Arctic and Antarctic. Phytochemical reports have identified diverse compounds, including xanthones,[4] depsides,[5] depsidones,[6] and fatty acids[7] from several *Pertusaria* species, including *Pertusaria amara*, *Pertusaria albescens*, *Pertusaria flavicans*, *Pertusaria pseudocorallina*, *Pertusaria truncate,* and other species from the genus found in China. However, the chemical and biological characteristics of *Pertusaria dactylina* in its Antarctic distribution have so far been poorly reported.[8] In continuation of our research focused on the chemistry and biochemistry of this Antarctic lichen, we isolated a new chlorinated-phenolic compound, together with 4 known mono phenyl derivatives. This paper reports on the isolation and structure elucidation of the new compound (Figure 1).

The ethyl acetate partition of the Antarctic lichen, *P. dactylina,* was repeatedly subjected to column chromatography (CC) on silica gel, RP-18 gel, Sephadex LH-20 gel, and semipreparative high performance liquid chromatography (HPLC) to afford a new chlorinated-phenolic compound **1**, along with 4 known compounds (**2**-**5**).

Compound **1** was obtained as a white amorphous powder. Its molecular formula of $C_{14}H_{20}ClO_4$ was deduced from an ion at m/z 287.1050 $[M+H]^+$ (calcd for $C_{14}H_{20}ClO_4$, 287.1050) in the positive HRFABMS (Supplemental Figure S1). The ^{1}H-NMR (Table 1; Supplemental Figures S2, S2-1 and S2-2) spectrum showed a singlet in the aromatic proton signal at δ_H 6.34 (s, H-5); a hydroxyl proton at δ_H 12.10 (s, 2-OH) shifted downfield due to the hydrogen bond with a carbonyl group; 4 methylene protons at δ_H 2.88 to 1.34 (H-1' to H-4') and a terminal methyl proton at δ_H 0.91 (*t*, J = 7.2 Hz, H-5'), indicating the presence of a pentyl group[9]; and 2 methoxy protons at δ_H 3.96 (s, 7-OCH_3) and δ_H 3.94 (s, 4-OCH_3). The ^{13}C-NMR spectrum (Supplemental Figure S3) revealed an ester carbonyl carbon at

[1]Unit of Research for Practical Application, Korea Polar Research Institute, Incheon, South Korea
[2]Department of Polar Sciences, University of Science and Technology, Incheon, South Korea
[3]Division of Life Sciences, Korea Polar Research Institute, KIOST, Incheon, South Korea

Corresponding Author:
Ui J. Youn, Division of Life Sciences, Korea Polar Research Institute, KIOST, Incheon 21990, South Korea.
Email: ujyoun@kopri.re.kr

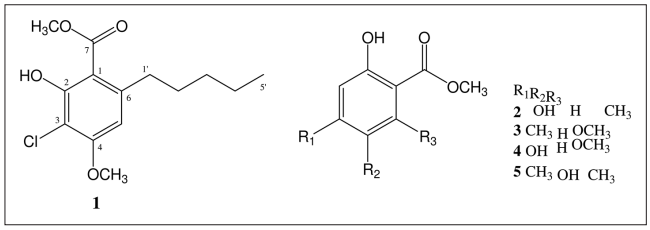

Figure 1. Chemical structures of compounds **1-5** isolated from *Pertusaria dactylina*.

δ_C 171.5 (C-7); 2 oxygen-bearing aromatic carbons at δ_C 159.8 (C-2) and δ_C 158.9 (C-4); 3 fully substituted aromatic carbons at [δ_C 146.3 (C-6), δ_C 107.4 (C-3), and δ_C 106.1 (C-1)], and a protonated aromatic carbon at δ_C 105.9 (C-5) assigned by heteronuclear single quantum coherence spectroscopy (HSQC) analysis (Supplemental Figures S4 and S4-1), including the pentyl group carbons at δ_C 37.2 (C-1'), 32.0 (C-3'), 31.7 (C-2'), 22.5 (C-4'), and δ_C 14.0 (C-5') confirmed by ^{1}H-^{13}C heteronuclear multiple bond correlation (HMBC) correlations (Supplemental Figure S5-2). The NMR data for **1** were quite similar to that of ethyl 3-chloro-2-hydroxy-4-methoxy-6-pentylbenzoate, a compound that has been reported previously,[9] except for a methoxy group at C-7 instead of ethoxy group of ethyl 3-chloro-2-hydr oxy-4-methoxy-6-pentylbenzoate.[9] The ^{1}H-^{13}C long range HMBC correlations (Supplemental Figures S5, S5-1 and S5-2) from δ_H 3.96 (s, 7-OCH$_3$) to δ_C 171.5 (C-7), 4-OCH$_3$/H-5 to C-4, and H-5 to C-3/C-1' supported the position of the methoxy group at the carbonyl carbon (C-7); the chlorine atom was attached at the C-3 position (Figure 2).[8] Thus, compound **1** was elucidated as a new compound, methyl-3-chloro-2-hydroxy-4-methoxy-6-pentylbenzoate.

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data for **1** ($CDCl_3$, δ, ppm, J/Hz).

	1	
C atom	δ_C	δ_H
1	106.1	
2	159.8	
3	107.4	
4	158.9	
5	105.9	6.34 (s)
6	146.3	
7	171.5	
1'	37.2	2.88 (m)
2'	31.7	1.54 (m)
3'	32.0	1.34 (m)
4'	22.5	1.34 (m)
5'	14.0	0.91 (t, J = 7.2)
4-OCH$_3$	56.2	3.83 (s)
7-COOCH$_3$	52.3	3.82 (s)

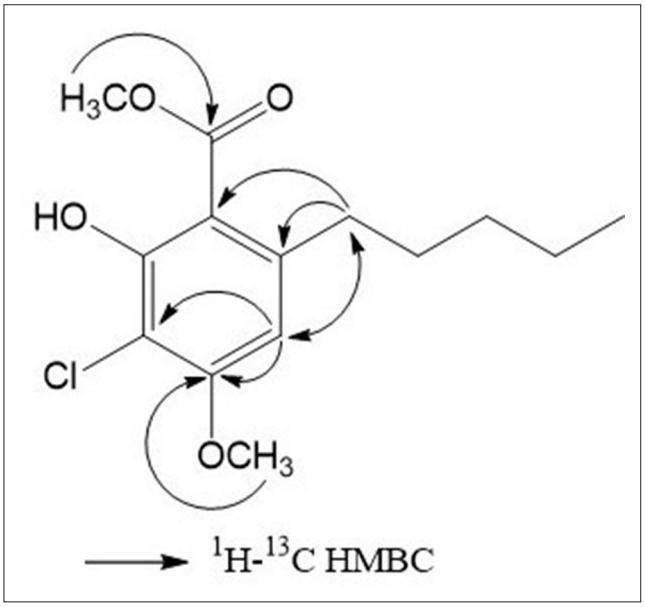

Figure 2. Key HMBC correlations of compound **1**.

The physical and spectral properties of the known compounds we isolated were compared with the published values, and identified as methyl 2,4-dihydroxy-6-methylbenzoate (**2**),[10] methyl 2-hydroxy-6-methoxy-4-methylbenzoate (**3**),[11] methyl 2,4-dihydroxy-6-methoxybenzoate (**4**),[11] and methyl 3,6-dihydr oxy-2,4-dimethylbenzoate (**5**).[12] To the best of our knowledge, compounds **2** to **5** have been isolated for the first time from this species.

Antimicrobial activities of compounds **1** to **5** against *Staphylococcus aureus* and *Candida albicans*, respectively, were evaluated. Among the isolated compounds, compound **1** exhibited a weak inhibitory effect against *C. albicans* with an IC_{50} value of 67 ± 7 μg/mL, when compared to the positive control constant (Nystatin, IC_{50} value of 1.4 ± 0.1 μg/mL). However, compounds **1** to **5** showed no inhibitory activity against *S. aureus* (Supplemental Table S1; Supplemental Figure S1).

Experimental

General

Optical rotations were measured on a Rudolph Research Autopol IV multiwavelength polarimeter. UV spectra were recorded on a Shimadzu PharmaSpec-1700 Ultraviolet (UV)-visible spectrophotometer. Infrared (IR) spectra were measured on a Bruker Tensor-27 spectrophotometer. 1D and 2D NMR spectra were recorded on a Bruker AVANCE (600 MHz) spectrometer. High-resolution electrospray ionization mass spectra (HREIMS) were obtained with an Agilent 6530 liquid chromatography quadrupole time-of-flight (LC-qTOF) High Mass Accuracy mass spectrometer operated in the positive- and negative-ion modes. Thin-layer chromatography

(TLC) was performed on silica gel 60 F_{254} (0.25 mm, Merck, Germany). Silica gel (230-400 mesh, Merck, Germany) and C-18 (YMC·GEL ODS-A, 12 nm, S-150 µm) were used for CC. Semipreparative HPLC was conducted on YL9100 HPLC system (Young Lin, South Korea) equipped with a UV/Vis detector using an Alltech reversed-phase YMC-Pak C-18 column (10 µm, 20 × 250 mm) with a flow rate of 2 mL/min.

Lichen Material

The lichen, *P. dactylina*, was collected in January 2017 from King George Island, Antarctica, (62°12'53.69" S; 58°55'23.87"W), and identified by Dr Ji Hee Kim and Miss Jae Eun So. A voucher specimen (no. Ant-053) was deposited at the Natural Product Chemistry Laboratory of the Korea Polar Research Institute.

Extraction and Isolation

An air-dried and powdered lichen, *P. dactylina* (80 g), was used for extraction by maceration in methanol (MeOH) (3 × 0.5 L) at room temperature. The solvent was concentrated in vacuo, yielding 3.0 g of a crude extract which was then suspended in distilled water (0.2 L) and extracted successively with *n*-hexane (2 × 0.2 L), ethyl acetate (EtOAc) (2 × 0.2 L), and *n*-butanol (2 × 0.2 L). The ethyl acetate partitions (1.2 g) were separated by CC over a silica gel column and eluted with Hex:EtOAc (90:10-50:50) to obtain 15 subfractions (Es1-Es15). The subfractions from Es4 to Es7 (250 mg) were combined based on their TLC patterns, subjected to a Sephadex LH-20 (30 g) gel CC using a solvent mixture (MeOH:H_2O, 50:50). They were then purified with HPLC on a semipreparative C-18 gel column, using an MeOH:H_2O mixture (60:40), and yielded **5** (2.0 mg, t_R 70 minutes), **2** (1.5 mg, t_R 78 minutes), and **4** (4.0 mg, t_R 83 minutes). The combined subfractions Es11 and Es12 (100 mg) were purified over a C-18 gel column, using MeOH:H_2O mixtures (10:90-80:20) as the solvent system, and yielded 5 subfractions (Es11r1-Es11r5). Subfraction Es11r2 (30 mg) was separated on a semipreparative C_{18} gel column by HPLC, using MeOH:H_2O mixtures (10:90-80:20) as the solvent system, yielded compounds **1** (2.0 mg, t_R 75 minutes) and **3** (3.0 mg, t_R 80 minutes).

Methyl-3-Chloro-2-Hydroxy-4-Methoxy-6-Pentylbenzoate (1)

White amorphous powder.
UV (MeOH) λ_{max} (log ε): 262 (4.0) nm.
^{1}H- (600 MHz, $CDCl_3$) and ^{13}C-NMR (150 MHz, $CDCl_3$) data, see Table 1.
HRFABMS: m/z 287.1050 $[M+H]^+$ (calcd for $C_{14}H_{20}ClO_4$, 287.1050)

Antibacterial Assays

The antimicrobial activities were tested using *S. aureus* KCTC 3881 (bacteria) and *C. albicans* KCTC 27242 (fungi) (Korean Collection for Type Cultures, Daejeon, Korea) in a 96-well plate. The cell culture was diluted up to 0.5 McFarland Standard with sterilized media. For *C. albicans*, the culture broth was 100 times more diluted before use. Each well was filled with 95 µL of culture broth. The compounds dissolved in DMSO were added until the final concentrations (0.5, 1, 2, 5, 10, 20, and 50 ug/mL), and the final volume of each well was 100 µL.[13] The plate was incubated at 25°C for 16 hours. Cell inhibition was measured at 600 nm (for *S. aureus*) and 530 nm (for *C. albicans*) using Multiskan GO Microplate Spectrophotometer (Thermo Scientific, Waltham, MA, United States). The IC_{50} value was calculated using an exponential trend line in Excel software (Microsoft, Redmond, WA, United States), and the values are mean ± standard errors of 3 determinations. Kanamycin and nystatin were used as positive controls against the bacterium and yeast, respectively.

Acknowledgments

We thank H.S. Shin, National Center for Inter University Research Facilities, Seoul National University, for the provision of the Mass Spectrometry Facility used in this study. We would like to thank Editage (www.editage.co.kr) for English language editing.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by a grant to the Korea Polar Research Institute, KOPRI, under a project (PE19210).

ORCID ID

Ui J. Youn https://orcid.org/0000-0001-9338-7934

Supplemental Material

Supplemental material for this article is available online.

References

1. Grube M, Cardinale M, de Castro JV, Müller H, Berg G. Species-Specific structural and functional diversity of bacterial communities in lichen symbioses. *Isme J*. 2009;3(9):1105-1115.
2. Schneider K, Resl P, Spribille T. Escape from the cryptic species trap: lichen evolution on both sides of a cyanobacterial acquisition event. *Mol Ecol*. 2016;25(14):3453-3468.
3. Boustie J, Tomasi S, Grube M. Bioactive lichen metabolites: alpine habitats as an untapped source. *Phytochem Rev*. 2011;10(3):287-307.
4. Santesson J, Wachtmeister CA. Chemical studies on lichens. XV. 2,4-Dichloro-6-methoxy-1,3-dihydroxy-8-methylxantho

ne (thiophaninic acid) from *Pertusaria flavicans*. *Arkiv Foer Kemi*. 1969;30(42):445-448.

5. Elix JA, Barclay CE, Wardlaw JH, Archer AW, Yu S-hua, Kantvilas G. Four new β-orcinol meta-depsides from *Pertusaria* and *Siphula* lichens. *Aust J Chem*. 1999;52(9):837-840.
6. Elix JA, Elix JA, Venables DA, Venables DA, Archer AW, Archer AW. Further new depsones from the lichen *Pertusaria truncata*. *Aust J Chem*. 1994;47(7):1345-1353.
7. Huneck S, Tønsberg T, Bohlmann F, substances L. (−)-Allo-pertusaric acid and (−)-dihydropertusaric acid from the lichen *Pertusaria albescens*. *Phytochemistry*. 1986;25(2):453-459.
8. Huneck S, Sainsbury M, Rickard TMA, Smith RIL. Ecological and chemical investigations of lichens from South Georgia and the maritime Antarctic. *J Hattori Bot Lab*. 1984;56:461-480.
9. Wolf V, Adeel M, Reim S, Villinger A, Fischer C, Langer P. Diversity-Oriented Synthesis of Chlorinated Arenes by One-Pot Cyclizations of 4-Chloro-1,3-bis(trimethylsilyloxy)buta-1,3-dienes. *European J Org Chem*. 2009;2009(33):5854-5867.
10. Basset J-F, Leslie C, Hamprecht D, White AJP, Barrett AGM. Studies on the resorcylates: biomimetic total syntheses of (+)-montagnetol and (+)-erythrin. *Tetrahedron Lett*. 2010;51(5):783-785.
11. Kikuchi H, Hoshikawa T, Kurata S, Katou Y, Oshima Y. Design and synthesis of Structure-Simplified derivatives of Gonytolide for the promotion of innate immune responses. *J Nat Prod*. 2016;79(5):1259-1266.
12. Ahmed Z, Fischer C, Spannenberg A, Langer P. Synthesis of functionalized *p*-dihydrobenzoquinones and *p*-benzoquinones based on [3+3] cyclizations of 1,3-bis-silyl enol ethers. *Tetrahedron*. 2006;62(20):4800-4806.
13. Jorgensen JH, Doern GV, Maher LA, Howell AW, Redding JS. Antimicrobial resistance among respiratory isolates of *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae* in the United States. *Antimicrob Agents Chemother*. 1990;34(11):2075-2080.