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Chemical constituents from the Antarctic lichen, *Stereocaulon caespitosum*

Ui Joung Youn[a,*], Jae Eun So[a,b], Ji Hee Kim[a], Se Jong Han[a,b], Hyun Park[b,c], Il Chan Kim[a], Jung Han Yim[a]

[a] Division of Life Sciences, Korea Polar Research Institute, KIOST, Incheon, 21990, Republic of Korea
[b] Department of Polar Sciences, University of Science and Technology, Incheon, 21990, South Korea
[c] Unit of Polar Genomics, Korea Polar Research Institute, Incheon, 21990, South Korea

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ABSTRACT

A phytochemical study of the methanol extract of the Antarctic lichen *Stereocaulon caespitosum* Redgr. led to the isolation of a tridepside (**1**), two depsides (**2** and **3**), a montagnetol derivative (**4**), and four mono-phenolic compounds (**5**–**8**). The structures of these compounds were confirmed by 1D- and 2D-nuclear magnetic resonance (NMR) experiments, as well as by comparison with published values. This is the first phytochemical study of *S. caespitosum*. In particular, compounds **1**, **3**, **4**, and **8** have been isolated for the first time from the genus *Stereocaulon* and the family Stereocaulaceae. The chemotaxonomic significance of the isolated compounds is discussed.

1. Subject and source

The genus *Stereocaulon* is widely distributed across the world, from tropical regions to the Arctic and Antarctic areas, with about 130 species in total. Among the species, *S. paschale* and *S. vulcani* are used in traditional medicine for the treatment of high blood pressure, diabetic symptoms, wounds and ulcers, and syphilis (Lavergne, 1989; Ismed et al., 2012). In addition, *S. alpinum*, collected from King George Island in Antarctica, shows interesting biological activities, such as anti-inflammatory (Lee et al., 2016), antioxidant (Bhattarai et al., 2013), and antibacterial (Bhattarai et al., 2013) activities, along with tyrosinase protein phosphatase 1B inhibitory (Seo et al., 2009), 5-lipoxygenase inhibitory (Ingolfsdottir et al., 1996), and cytotoxic properties (Seo et al., 2008) (see Fig. 1).

Stereocaulon caespitosum Redgr. (Stereocaulaceae) is a fruticose lichen with a height of 2–5 mm that grows on stony surfaces and glacial outwash debris, which are dominated by *Acaena magellanica* and *Syntrichia robusta*. This lichen is mainly distributed in New Zealand, Tasmania Island of Australia, and the South Georgia Island in the Antarctic (Øvstedal and Smith, 2001).

The lichen, *S. caespitosum*, 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-061) was deposited at the Natural Product Chemistry Laboratory of the Korea Polar Research Institute.

2. Previous work

In previous studies, about 75 compounds, including alkamides (Ingolfsdottir et al., 1997), benzofurans (Claudia et al., 2017), carbohydrates (Baron et al., 1988; Yokota and Shibata, 1978), depsidones (Ismed et al., 2012; Ingolfsdottir et al., 1996; Seo et al., 2009), a depsipeptide (Seo et al., 2008), pseudodepsidones (Seo et al., 2009; Claudia et al., 2017), depsides (Ismed et al., 2017), mono-phenolic compounds (Vila et al., 2008; Gonzalez et al., 1992), steroids (Gonzalez et al., 1992), and triterpenes (Konig and Wright, 1999), have been reported from 40 *Stereocaulon* species. However, most of the species have been poorly investigated so far. In particular, there are no chemical or biological studies on *S. caespitosum*. Herein, we report first time secondary metabolites that were obtained from the Antarctic lichen, *S. caespitosum*.

3. Present study

The air-dried and powdered lichen, *S. caespitosum* (100 g), was extracted by maceration in methanol (MeOH) (3 × 0.5 L) at room temperature. The solvent was concentrated in vacuo to yield 5 g of a crude extract, which was then suspended in distilled water (0.2 L) and extracted successively with *n*-hexane (2 × 0.5 L), ethyl acetate (EtOAc) (2 × 0.5 L), and *n*-butanol (2 × 0.5 L). The EtOAc extracts (2.3 g) were separated by column chromatography (CC) over a C_{18} gel column and eluted with MeOH:H_2O (10:90–100% MeOH) to obtain 18 subfractions (ER1 to ER18).

* Corresponding author.
E-mail address: ujyoun@kopri.re.kr (U.J. Youn).

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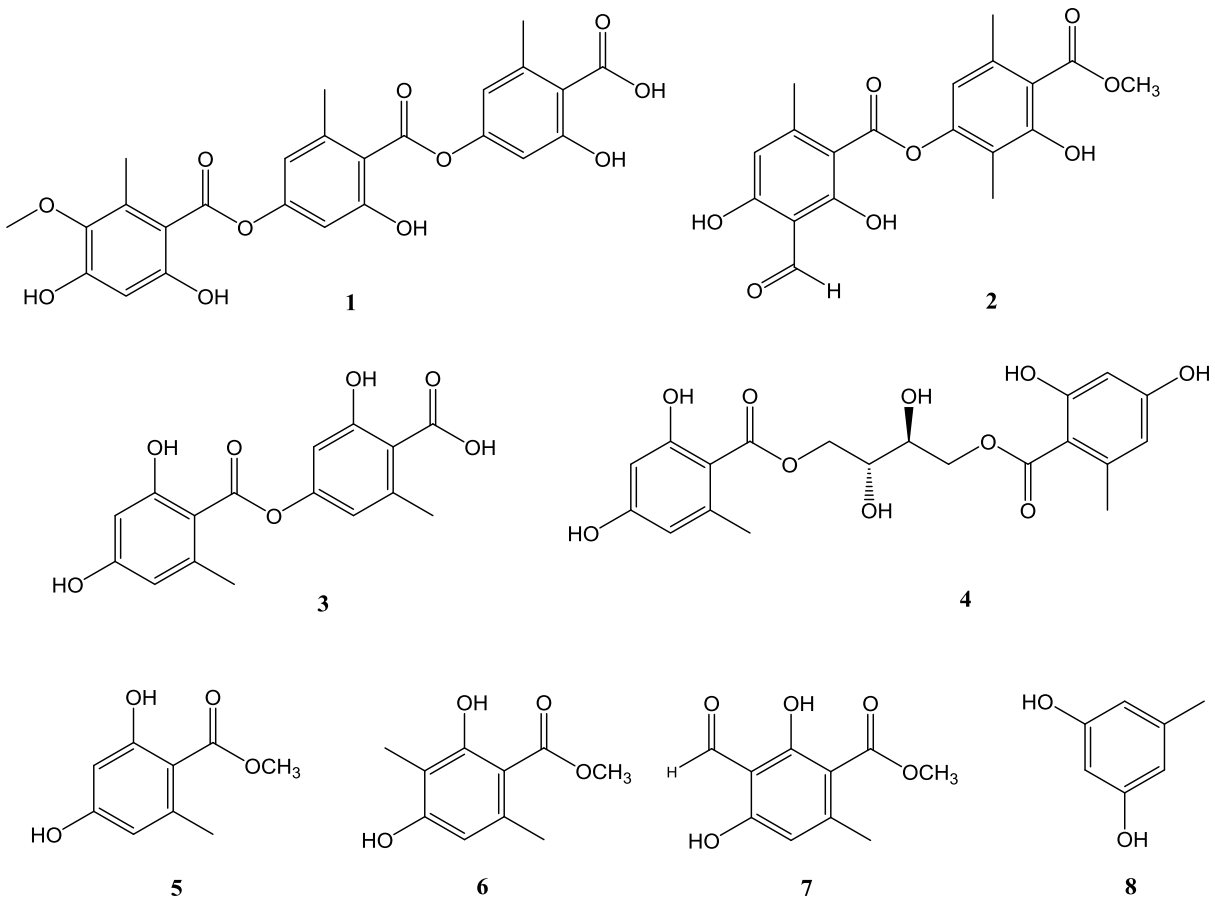

Fig. 1. Chemical structures of compounds **1**–**8**.

Subfraction ER1 (50 mg) was subjected to a second round of chromatography with an open C_{18} (50 g) gel column using solvent mixtures (MeOH:H_2O, 10:90–100% MeOH), and purified by HPLC on a semi-preparative RP-18 gel column, using MeOH:H_2O mixtures, from 20:80 to 90:10, as the solvent system, to yield **4** (1.5 mg, t_R 80 min) and **8** (1 mg, t_R 95 min). Subfraction ER14 (100 mg) was purified over a silica gel column, using $CHCl_3$:MeOH mixtures (from 100:0 to 50:50) as the solvent system, to yield four subfractions (ER14S1 to ER14S4). The combined subfractions ER14S2 and ER14S3 (30 mg) were subjected to separation on a semi-preparative RP-18 column by HPLC, using MeOH:H_2O mixtures (from 50:50 to 80:20) as the solvent system, to yield compounds **3** (2.5 mg, t_R 85 min) and **5** (6 mg, t_R 90 min). Atranorin (**2**, 10 mg) was obtained as a white amorphous powder by purification with 100% chloroform ($CHCl_3$) from subfraction ER12 (50 mg). Compounds **6** (5 mg, t_R 100 min) and **7** (3.5 mg, t_R 105 min) were isolated from subfraction ER13 (40 mg) by a semi-preparative RP-18 column and HPLC methods, using MeOH:H_2O, from 50:50 to 80:20, as the solvent system. Fraction ER18 (170 mg) was subjected to chromatography on a Sephadex LH-20 gel (100 g) column and eluted with a MeOH:H_2O (from 0:100 to 50:50) solvent system, to give three subfractions (ER18L1 to ER18L3). Subfraction ER18L1 (60 mg) was purified by semi-preparative HPLC on an RP-18 column, using MeOH:H_2O solvent mixtures (from 60:40 to 90:10), to yield **1** (8 mg, t_R 87 min).

The compounds were identified as 5-*O*-methylhiascic acid (**1**) (Elix et al., 1981), atranorin (**2**) (Vu et al., 2015), lecanoric acid (**3**) (Hamada and Ueno, 1990), orsellinylmontagnetol C (**4**) (Duong et al., 2017), methyl orsellinate (**5**) (Seo et al., 2009), atraric acid (**6**) (Hylands and Ingolfsdottir, 1985), methyl haematommate (**7**) (Huneck and Yoshimura, 1996), and orcinol (**8**) (Lopes et al., 2008), by comparison of their physical and spectral properties with published values.

4. Chemotaxonomic significance

The current study reports the isolation and structure elucidation of a tridepside (**1**), two didepsides (**2** and **3**), a montagnetol derivative (**4**), and mono-phenolic compounds (**5**–**8**) from the Antarctic lichen, *S. caespitosum*. This was the first time that all these compounds were isolated from this species.

Tridepsides are known as important taxonomic markers in Umbilicariaceae (Narui et al., 1998) and Parmeliaceae families, and as bioactive metabolites that possess antidiabetic, anti-obesity, anti-proliferative, cytotoxic, and human leukocyte elastase and human cytomegalovirus protease inhibitory activities.

In particular, hiascic acid derivatives have been reported as characteristic metabolites in the genus *Parmelia*. Although, 5-*O*-methylhiascic acid (**1**) has been reported in *P. damaziana* (Elix et al., 1981), this tridepside (**1**) was isolated for the first time from the genus *Stereocaulon* and the family Stereocaulaceae in this study.

Didepsides, depsidones, and pseudodepsidones are characteristic secondary metabolites in various lichens, including the *Stereocaulon* genus. Previous studies have reported important chemical markers: lobaric acid, atranorin, and stictic acid, from the *Stereocaulon* species (Ingolfsdottir et al., 1998; Ismed et al., 2017; Miyagawa et al., 1997; Luis Vila et al., 2004). Among the compounds, atranorin (**2**) is a common lichen metabolite that is found in various *Stereocaulon* species: *S. alpinum* (Ingolfsdottir et al., 1998), *S. argus* (Huneck, 1974), *S. azeroum* (Gonzalez et al., 1992), *S. colensoi* (Fox et al., 1970), *S. curtatum* (Hamada and Ueno, 1990), *S. evolutum* (Vu et al., 2015), *S. montagneanum* (Ismed et al., 2017), *S. myriocarpum* (Huneck, 1974), *S. ramulosum* (Vila and Gimenez, 1999), *S. strictum* (Huneck, 1974), *S. tomentosum* (Luis Vila et al., 2004), and *S. vesuvianum* (Konig and

Wright, 1999), and it is also found in *S. caespitosum* as a major secondary metabolite. Lecanoric acid (**3**), another didepside derivative, has been reported as a secondary metabolite of various lichen genera, such as *Acroscyphus*, *Parmotrema*, *Melanelia*, *Ramalina*, *Lethariella*, *Parmelia*, *Thamnolia*, and *Umbilicaria*. Meanwhile, **3** has been isolated for the first time from the mycobiont *S. curtatum* (Hamada and Ueno, 1990), but it has not been found in a lichenized body from the *Stereocaulon* species to date.

As a polyol, erythritol is assumed to be produced by the photobiont (Nash, 2008), and it has been determined that its substitution with orsellinic acid produces large amounts of D-montagnetol in *Rocella* lichens (Duong et al., 2017). In addition, orsellinylmontagnetols, composed of two orsellinyl groups and an erythritol, have only been reported in a species, *R. montagnei* (Duong et al., 2017). In this study, oresellinylmontagnetol C (**4**) was found in the Antarctic lichen, *S. caespitosum*, for the first time.

Mono-phenyl compounds, such as methyl orsellinate (**5**), atraric acid (**6**), methyl haematommate (**7**), and orcinol (**8**), have been commonly found in most *Stereocaulon* species; they are also important primary constituents for the formation of secondary lichen metabolites, such as depsidones, pseudodepsidones, depsides, and other polyphenolic compounds.

In conclusion, atranorin (**2**) and mono-phenolic compounds (**5–7**) exist widely in the *Stereocaulon* species, and these could be used to verify the chemotaxonomic relationships between the related *Stereocaulon* species and the *Stereocaulaceae* genus. On the other hand, 5-*O*-methylhiascic acid (**1**), lecanoric acid (**3**), and orsellinylmontagnetol C (**4**), including orcinol (**8**), have not been found in this genus or in the family Stereocaulaceae. Therefore, these chemical metabolites could be important chemotaxonomic markers for the identification of *S. caespitosum*.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bse.2018.07.004.

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