Biochemical Systematics and Ecology 106 (2023) 104581

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Chemical constituents from the Antarctic lichen *Usnea aurantiaco-atra* and their chemotaxonomic significance

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ARTICLE INFO

Keywords:
Usnea aurantiaco-atra
Antarctic lichen
Dibenzofuran
Sterol
p-Terphenyl

ABSTRACT

Chemical investigation of the Antarctic lichen *Usnea aurantiaco-atra* led to the isolation and identification of eight compounds, including a dibenzofuran derivative (**1**), three phenolics (**2**, **7**, **8**), a *p*-terphenyl (**3**), and three sterols (**4**–**6**). The structures of the isolated compounds (**1**–**8**) were elucidated by ^{1}H and ^{13}C NMR spectroscopic analyses and comparisons with previously reported data. All compounds (**1**–**8**) were isolated from this species for the first time. The current study is also the first report of the identification of two compounds, pentamethoxy-*p*-terphenyl and 9,11-dehydroergosterol peroxide, from the *Usnea* genus. Meanwhile, the chemotaxonomic value of all isolates is also discussed in this paper.

1. Subject and source

The *Usnea* genus (Parmeliaceae) is a group of more than 360 lichen species that are widely distributed in both Eastern and Western countries. Many species in this genus were found to be important folk medicine worldwide (Prateeksha et al., 2016). People have traditionally consumed them to treat diseases such as diarrhea, ulcer, urinary infection, tuberculosis, pneumonia, stomachache, and cattle fungal diseases (Gómez-Serranillos et al., 2014; Prateeksha et al., 2016). Some other uses of this genus for antiseptic, antiviral, and antifungal agents, as well as for fever control, pain relief, wound healing, and hair growth have also been reported (Gómez-Serranillos et al., 2014; Prateeksha et al., 2016). Additionally, various studies have described the pharmacological activities of methanol, acetone, and aqueous extracts from *Usnea* species; these extracts provide antioxidant (Behera et al., 2006), antibacterial (Behera et al., 2005; Tatipamula and Annam, 2022), hepatoprotective (Verma et al., 2008), antiulcerogenic (Halici et al., 2005), antigenotoxic (Ceker et al., 2015), lipid regulatory (Zhu et al., 2017), and anticancer functions (Tang et al., 2020).

In this study, the lichen *U. aurantiaco-atra* was collected in February 2021 from King George Island, Antarctica, and identified by Dr. Ji Hee Kim and Miss Jae Eun So. A voucher specimen was deposited at the Natural Product Chemistry Laboratory of the Korea Polar Research Institute.

2. Previous work

There were no previous reports available on the chemical composition of *U. aurantiaco-atra*. Previous investigations on the genus *Usnea* led to the isolation of various secondary metabolites, including depsides (Honda et al., 2010), depsidones (Brandão et al., 2013; Lohezic-Le Devehat et al., 2007), mono-phenolics (Mallavadhani et al., 2004), steroids (Mallavadhani et al., 2004), and benzofurans (Honda et al., 2010). Particularly, a dibenzofuran, usnic acid, was reported as a characteristic constituent of the genus *Usnea* (Cansaran et al., 2006). Herein, we describe for the first time the secondary metabolites obtained from the Antarctic lichen, *U. aurantiaco-atra*.

3. Present study

The air-dried and powdered lichen *U. aurantiaco-atra* (500 g) was extracted repeatedly with methanol (MeOH) (3 L x 3 times) at room

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https://doi.org/10.1016/j.bse.2022.104581
Received 21 September 2022; Received in revised form 11 December 2022; Accepted 29 December 2022
Available online 30 December 2022

temperature for three days. The crude extract (28.5 g) was concentrated under reduced pressure by a rotary evaporator to give a residue. The residue was suspended in distilled water and sequentially partitioned with *n*-hexane, chloroform ($CHCl_3$), ethyl acetate (EtOAc), and *n*-butanol (BuOH). Based on thin-layer chromatography (TLC) analysis, the hexane and EtOAc layers were selected for further isolation.

The hexane layer was dried (6.2 g) and adsorbed onto silica gel CC. The gravity elution was performed with the gradient solvent system of hexane with increasing amounts of EtOAc (hexane:EtOAc, 95:5–0:100), followed by mixtures of $CHCl_3$ and MeOH ($CHCl_3$:MeOH, 100:0–50:50) to yield 13 fractions (H1–H13). Fraction H4 (59.0 mg) was purified by HPLC eluted with hexane:EtOAc (90:10, 3 mL/min) using a normal-phase YMC-Pack SIL-HG column (250 × 20 mm I.D., 10 μm) to obtain compounds **2** (t_R 59.6 min, 4.0 mg) and **3** (t_R 69.3 min, 1.0 mg). Fraction H6 (320.0 mg) was separated into three subfractions (H6.1 to H6.3) using silica gel CC and a mixture of hexane with increasing amounts of EtOAc as the eluting solvent system (hexane:EtOAc 90:10–70:30). Subfraction H6.1 (74.0 mg) was then subjected to C-18 CC and eluted with methanol solvent (MeOH:H_2O, 80:20–100:0) to afford eight subfractions (H6.1.1 to H6.1.8). Purification of H6.1.7 (28.0 mg) was conducted by HPLC (hexane:EtOAc, 30:70, 2 mL/min) using the normal-phase YMC-Pack SIL-HG column (250 × 20 mm I.D., 10 μm), which resulted in the isolation of **4** (t_R 40.4 min, 7.2 mg). Subfraction H6.2 (144.0 mg) was loaded on C-18 CC and then eluted with a mixture of MeOH and water (MeOH:H_2O, 90:10) to give four subfractions (H6.2.1 to H6.2.4). H6.2.3 (19.0 mg) was further isolated using HPLC (hexane: EtOAc, 30:70, 2 mL/min) equipped with the normal-phase YMC-Pack SIL-HG column (250 × 20 mm I.D., 10 μm) to yield **5** (t_R 47.6 min, 9.5 mg). Finally, compound **6** (t_R 50.1 min, 64.7 mg) was collected after purifying H6.2.4 (85.0 mg) using HPLC (hexane:EtOAc, 25:75, 2 mL/min) equipped with the normal-phase YMC-Pack SIL-HG column (250 × 20 mm I.D., 10 μm).

The EtOAc layer (12.0 g) was subjected to silica gel CC and eluted with the solvent system of hexane with increasing amounts of EtOAc (hexane:EtOAc, 90:10–0:100), followed by mixtures of $CHCl_3$ and MeOH ($CHCl_3$:MeOH, 100:0–50:50) to yield nine fractions (E1 – E9). Fraction E1 (2.6 g) was fractionated by silica gel CC, with the eluting solvent MeOH in $CHCl_3$ ($CHCl_3$:MeOH, 95:5–30:70) to yield four fractions (E1.1 – E1.4). Fraction E1.2 was crystallized and washed in MeOH to give compound **1** (867.0 mg). Fraction E1.4 (155.0 mg) was loaded and eluted with a mixture of MeOH and H_2O (MeOH:H_2O, 80:20) through a Sep-Pak Cartridge packed with C-18 particles to give two subfractions, E1.4A and E1.4B. Subfraction E1.4A (62.0 mg) was injected into the HPLC system equipped with a reserved-phase Alltech YMC-Pak Pro C18 column (10 μm, 20 × 250 mm) and eluted by a gradient solvent system of MeOH and H_2O (MeOH:H_2O, 70:30 to 100:0, 3 mL/min) to afford five subfractions (E1.4A.1 – E1.4A.5). Subfraction E1.4A.1 (5.0 mg) was further purified on HPLC (hexane:EtOAc, 50:50, 3 mL/min) using a normal-phase YMC-Pack SIL-HG column (S-10 μm, 12 nm, 20 × 250 mm) to obtain **7** (t_R 24.4 min, 3.0 mg). Subfraction E1.4A.2 (2.0 mg) was isolated by HPLC (hexane:EtOAc, 5:5, 3 mL/min) using a normal-phase YMC-Pack SIL-HG column (S-10 μm, 12 nm, 20 × 250 mm) to give **8** (t_R 23.7 min, 0.9 mg).

The structures of eight isolates were identified by spectroscopic analysis using ^1H and ^{13}C NMR, matching with data described in previously reported studies: (+)-usnic acid (**1**) (Rashid et al., 1999), methyl 2,4-dihydroxy-3,6-dimethylbenzoate (**2**) (Wang et al., 2005), pentamethoxy-*p*-terphenyl (**3**) (Chikako et al., 1976), brassicasterol (**4**) (Shukla et al., 2004), 9,11-dehydroergosterol peroxide (**5**) (Chen et al., 2009; Wang et al., 2020), ergosterol peroxide (**6**) (Chen et al., 2009; Wang et al., 2020), methyl orsellinate (**7**) (Lopes et al., 2008) and ethyl orsellinate (**8**) (Lopes et al., 2008) (Fig. 1).

4. Chemotaxonomic significance

The present study describes eight compounds isolated for the first time from the Antarctic lichen *U. aurantiaco-atra*: a benzofuran (**1**), three mono-phenolics (**2**, **7**, and **8**), a *p*-terphenyl (**3**), and three sterols (**4**–**6**). Based on an extensive literature survey, we present the first report of compound **3**, a rare substance, and an ergosterol derivative (**5**) in the genus *Usnea*, which can serve as potential chemotaxonomic markers to distinguish *U. aurantiaco-atra* from other intrageneric species.

The presence of usnic acid (**1**), a well-known compound possessing various biological activities (Gómez-Serranillos et al., 2014), is a

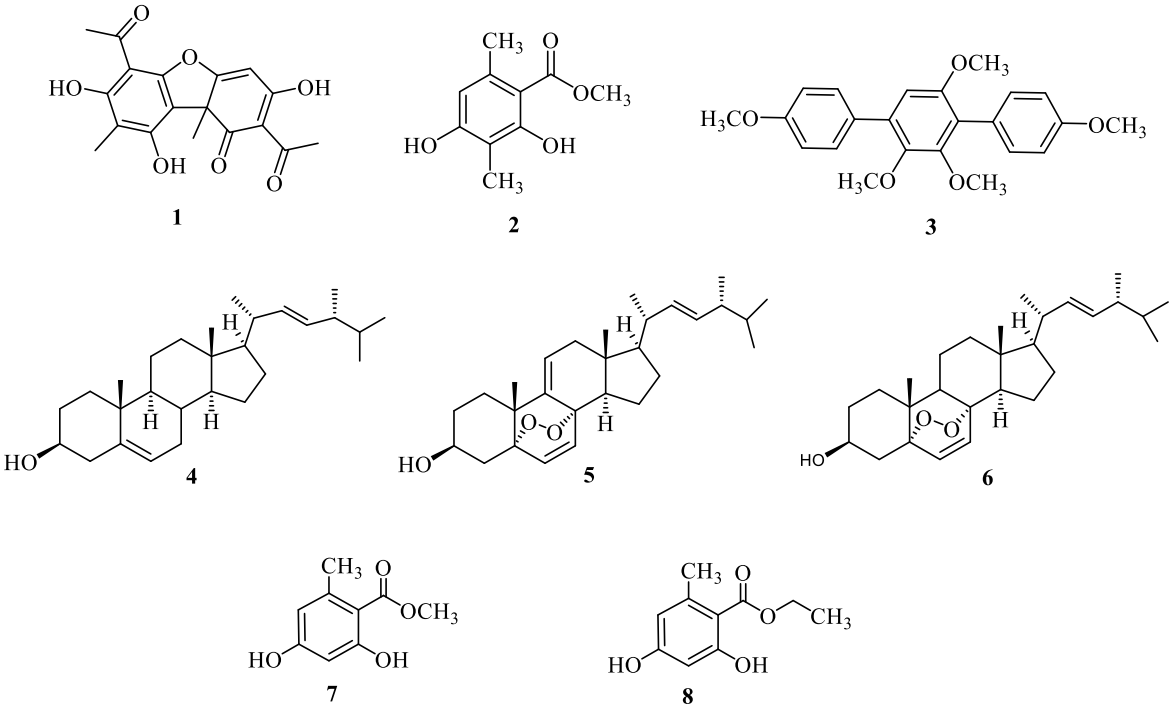

Fig. 1. Compounds **1**–**8** isolated from the methanol extract of *U. aurantiaco-atra*.

characteristic of lichen species with a yellow-green upper cortex, including those in the genera *Alectoria*, *Evernia*, *Flavoparmelia*, and *Usnea* (Gómez-Serranillos et al., 2014). Previous studies on *Usnea* have confirmed that the compound is abundant in *Usnea* species: *U. longissimi* (Yu et al., 2016), *U. sp.* in Sri Lanka (Kathirgamanathar et al., 2005), *U. florida*, *U. barbata*, *U. rigida*, *U. subflorida*, and *U. hirta* (Cansaran et al., 2006). Thus, usnic acid obtained in the current study agrees with previous reports and supports the taxonomic of *U. aurantiaco-atra* in the genus.

Compounds **2**, **7**, and **8** belong to the mono-phenolic group and often appear as lichen metabolites – the orsellinate derivatives serve as building blocks for the biosynthesis of more complex lichen substances (Calcott et al., 2018; Ismed et al., 2018). Atraric acid (**2**) has been previously reported in *U. longissima* (Ullah et al., 2019), *U. baileyi* (Van Nguyen et al., 2018), *U. undulata* (Sultana and Afolayan, 2011), and *U. articulata* (Lohezic-Le Devehat et al., 2007). Methyl orsellinate (**7**) was identified from *U. ceratina* (Bui et al., 2022), *U. diffracta* (Qi et al., 2009), and *U. undulata* (Do et al., 2019). Although ethyl orsellinate (**8**) can be found in various lichens, this compound has only been described in *U. diffracta* (Qi et al., 2009) within the genus. These findings provide more evidence for the position of *U. aurantiaco-atra* belongs to the *Usnea* genus and suggest the close relationship between it and *U. diffracta*.

Terphenyls, interesting compounds possessing cytotoxic, anti-inflammatory, antibacterial, and antioxidant properties, are rare components that appear to be restricted to fungi and lichens (Ranković and Kosanić, 2015; Valeria et al., 2003). Notably, there are only a few reports on the isolation of these compounds from lichens. Pentamethoxy-*p*-terphenyl (**3**) has only been found in two fungi: *Aspergillus candidus* and *Floricola striata* (Chikako et al., 1976; Xu et al., 2018). Therefore, the study reports for the first time the discovery of pentamethoxy-*p*-terphenyl in *Usnea*, broadens the distribution of this compound in nature and enhances understanding of the chemical composition among the genus. The current result suggests that this terphenyl can be used to differentiate *U. aurantiaco-atra* from other *Usnea* species.

Ergosterol derivatives, brassicasterol (**4**), 9,11-dehydroergosterol peroxide (**5**), and ergosterol peroxide (**6**), are common sterols but rarely can obtained from lichens. In the genus *Usnea*, compound **4** has only been found in *U. antarctica* (Tabacchi et al., 1987) and compound **6** only in *U. articulata* (Lohezic-Le Devehat et al., 2007), but compound **5** has never been isolated from the genus *Usnea*, the latter only has been found in one lichen, *Parmelia omphalodes* (Tabacchi et al., 1987). Hence, while the presence of compound **4** and **6** implies a close relationship between *U. antarctica*, *U. articulata* and *U. aurantiaco-atra* within the genus, the discovery of sterol **5** for the first time from *Usnea* species enriches the chemical profile of this genus and suggests the use of compound **5** as a potential way to distinguish *U. aurantiaco-atra* from other intrageneric species.

In summary, we isolated and identified eight compounds, namely a benzofuran (**1**), three phenolic compounds (**2**, **7**, **8**), a terphenyl (**3**), and three sterols (**4**–**6**) from *U. aurantiaco-atra* for the first time in the current study. Among them, the presence of usnic acid (**1**), three phenolics, and sterols (**4** and **6**) corresponds with the congeneric profile and confirms the taxonomic position of *U. aurantiaco-atra* in the genus. In contrast, compounds **3** and **5** have never been isolated from other *Usnea* species and comprise distinctive components of *U. aurantiaco-atra*. Thus, this study extends our knowledge of new chemical information for the genus *Usnea* and the Antarctic lichen, *U. aurantiaco-atra,* suggesting that compounds **3** and **5** could be chemotaxonomic markers for the identification of *U. aurantiaco-atra* based on their narrow distribution within the *Usnea* genus.

Author statement

Conceptualization Kim-Hoa Phi, Ui Joung Youn Methodology Kim-Hoa Phi Validation Jae Eun So, Ji Hee Kim Writing – Original Draft Kim-Hoa Phi Writing – Review & Editing Kim-Hoa Phi, Ui Joung Youn, Seulah Lee Visualization, Investigation, Data Curation Kim-Hoa Phi, Man Hyung Koo Resources Ui Joung Youn, Dockyu Kim Supervision Ui Joung Youn, Seulah Lee Project administration Jin-Hyoung Kim, Jun Hyuck Lee Funding acquisition Jin-Hyoung Kim, Jun Hyuck Lee.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgments

This work was part of a project titled “Development of potential antibiotic compounds using polar organism resources (20200610, KOPRI Grant PM22030)”, funded by the Ministry of Oceans and Fisheries, Korea. This study was also supported by the Korea Polar Research Institute (KOPRI; grant number PE22160).

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