NATURAL PRODUCT RESEARCH
2020, VOL. 34, NO. 18, 2573–2580
https://doi.org/10.1080/14786419.2018.1544982

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Antimicrobial secondary metabolites of an endolichenic *Aspergillus niger* isolated from lichen thallus of *Parmotrema ravum*

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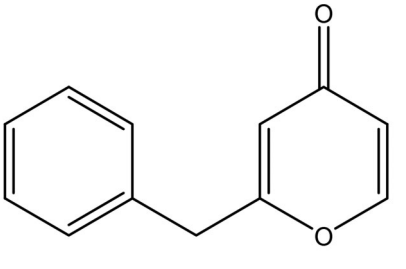
ABSTRACT

A new 6-benzyl-γ-pyrone (**1**), named aspergyllone was isolated from the culture filtrates of an endolichenic fungus *Aspergillus niger* Tiegh, obtained from lichen thallus *Parmotrema ravum* (Krog & Swinscow) Serus, collected in India. **1** was isolated for the first time from an endolichenic fungus together with six other known metabolites identified as aurasperones A (**2**) and D (**3**), asperpyrone A (**4**), fonsecinone A (**5**), carbonarone A (**6**) and pyrophen (**7**). The compounds were tested against a panel of human, plant, food borne and fish pathogens. Aspergyllone showed strong selective antifungal activity against *Candida parapsilosis* (Ashford) Langeron & Talice, with an IC_{50} of 52 μg/mL. Aurasperone A and pyrophen showed moderate to strong antimicrobial activity inhibiting seven different test pathogens, being pyrophen active with IC_{50} ranging from 35 to 97 μg/mL.



1, Aspergyllone

ARTICLE HISTORY
Received 3 October 2018
Accepted 2 November 2018

KEYWORDS
Endolichenic fungus; *Parmotrema ravum*; *Aspergillus niger*; aspergyllone; antimicrobial activity

1. Introduction

Lichen thalli are known to be colonized by numerous asymptomatic and cryptic micro fungi that live in close association with the photobiont. These diverse groups of fungi,

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Supplemental data for this article can be accessed 10.1080/14786419.2018.1544982.

which reside in the interior of a lichen thallus, have been termed as 'endolichenic fungi' (Li et al. 2007; Paranagama et al. 2007). Thus, endolichenic fungi are similar to plant endophytes and established themselves as endosymbiont. Although they have been characterized from a limited number of lichen species and geographic areas, current evidence suggests that like endophytes, endolichenic fungi are horizontally transmitted, form highly localized infections, and include abundant taxa belonging to diverse classes, orders and families within the Ascomycota (Pezizomycotina) (Arnold et al. 2009; Shaaban et al. 2012; Tripathi et al. 2014). In the recent years, endolichenic fungi have been identified as new sources of bioactive secondary metabolites (Kellogg and Raja 2017, Cimmino et al. 2018). In fact, several metabolites obtained from endolichenic fungi have shown promising antibacterial, antifungal and anticancer properties (Ding et al. 2009; Wang et al. 2010; Wang et al. 2013; Wu et al. 2015). Although there are reports on endolichenic fungi from India (Suryanarayan et al. 2005; Suryanarayanan and Thirunavukkarasu 2017) nevertheless, studies on bioactivity and chemistry of their secondary metabolites have not been thoroughly explored and still remain unstudied.

In this manuscript the endolichenic fungus from lichen thallus of *Parmotrema ravum* (Krog & Swinscow) Serus collected from Similipal Biosphere Reserve, India, was investigated and an endolichenic fungus *Aspergillus niger* Tiegh, representing fungal taxa of class Ascomycetes, was also isolated. Similar studies were made from various lichen taxa resulting into isolation of several endolichenic fungi especially of the fungi belonging to phylum Ascomycota (Petrini et al. 1990; Tripathi and Joshi 2015). In many instances researchers working on these fungi focused of secondary metabolites as they have been identified as a new avenue for discovery of bioactive secondary metabolite chemistry in natural products research (Kellogg and Raja 2017).

In our earlier investigation the occurrence of endolichenic fungi from lichen thalli of Similipal Biosphere Reserve India and their antimicrobial potentials (Padhi and Tayung 2015; Padhi et al. 2017; Padhi et al. 2018) were reported. This paper reports the isolation, antimicrobial activity and chemical characterization of a new 6-benzyl-γ-pyrone, named aspergyllone, as well as on the identification of other two known 6-benzyl-γ-pyrones and four known bi[benzo[*g*]chromenyl]-4,4'-diones.

2. Results and discussion

The organic extract obtained from the *Aspergillus* sp. culture filtrates was purified by bioassay-guided fractionation as reported in Experimental section, yielding seven pure metabolites (**1**–**7**, Figure 1). Metabolites **2**–**7** were identified comparing their spectroscopic data (essentially ^{1}H-NMR and ESI-MS) with those reported in literature as aurasperones A (Fang et al. 2016) and D (Ghosal et al. 1979), asperpyrone A, fonsecinone A (Fang et al. 2016), carbonarone A (Zhang et al. 2007) and pyrophen (Zhang et al. 2010). Furthermore the absolute configuration of pyrophen was confirmed comparing its specific optical rotation data ($[\alpha]^{25}_{D}$: −14.2 ($c = 0.1$ $CHCl_3$) with that reported in literature ($[\alpha]^{25}_{D}$: −13.8 ($c = 0.1$ $CHCl_3$)) (Zhang et al. 2010).

Also a new benzyl-γ-pyrone, named aspergyllone (**1**, Figure 1) was isolated as an amorphous solid from the same fungal organic extract. Its molecular formula was determined as $C_{12}H_{10}O_2$ based on the HRESIMS and was consistent with eight

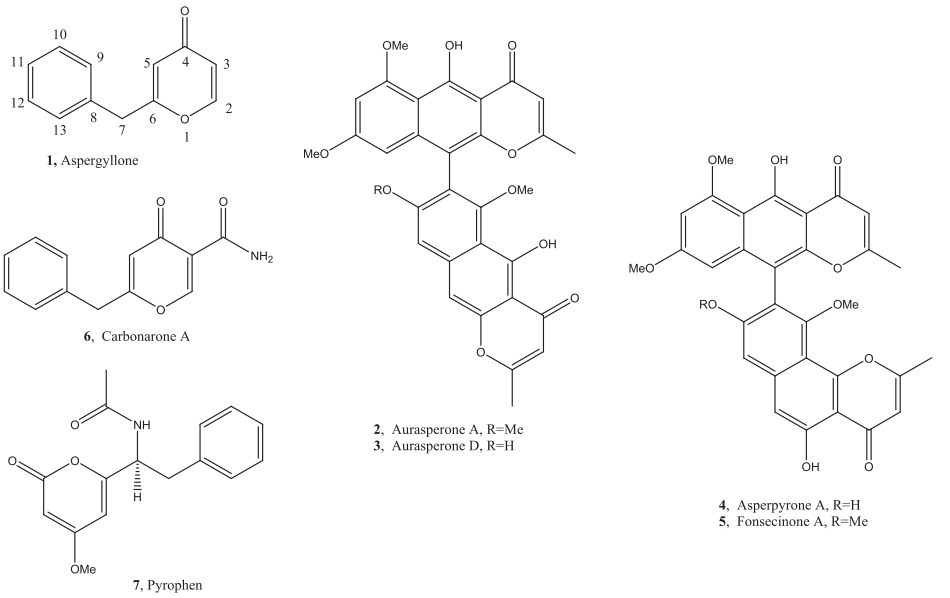

Figure 1. Structures of aspergyllone, aurasperones A and D, asperpyrone A, fonsecinone A, carbonarone A and pyrophen (**1**–**7**, respectively).

Table 1. ^{1}H, ^{13}C NMR and HMBC data of aspergyllone (**1**) in $CDCl_3$[a,b].

Position	δC[c]	δH (*J* in Hz)	
2	154.7 CH	7.66 d (5.8)	
3	117.0 CH	6.26 dd (5.8, 2.5)	
4	178.8 C		H-2
5	115.1 CH	6.13 d (2.5)	H-2, H-7
6	168.8 C		H-2, H-7
7	40.1 CH_2	3.81 s	
8	134.9 C		H-7
9,13	129.5 CH	7.26 dd (7.5, 1.6)	H-7, H-10,12
10,12	129.5 CH	7.35 t (7.5)	H-9,13
11	128.0 CH	7.33 tt (7.5, 1.6)	

[a]The chemical shifts are in δ values (ppm) from TMS.
[b]2D ^{1}H, ^{1}H (COSY) ^{13}C, ^{1}H (HSQC) NMR experiments delineated the correlations of all the protons and the corresponding carbons.
[c]Multiplicities were assigned by DEPT spectrum.

hydrogen deficiencies. The first preliminary investigation of its ^{1}H and ^{13}C NMR (Table 1) showed that **1** was closely related to carbonarone A (**6**) by comparison of their spectroscopic data (Zhang et al. 2007). The IR spectra are very similar but that of **1** lacks the typical amide bands (C=O and NH_2) (Nakanishi and Solomon 1977). The ^{1}H NMR spectrum of **1** differed from that of carbonarone A for the signal system of γ-pyrone ring. In fact, the ^{1}H NMR spectrum of **1** (Table 1) showed the significant upfield shift (Δδ 1.07) of H-2 which appeared as a doublet ($J = 5.8$ Hz) at δ 7.66 and the presence of H-3, which appeared as a double doublet ($J = 5.8$ and 2.5 Hz) at δ 6.26, being coupled in the COSY spectrum (Berger and Braun 2004) with both H-2 and H-5. Instead H-2 in carbonarone A was a singlet resonating at δ 8.73 (Pretsch et al. 2000). The ^{13}C NMR spectrum of **1** differed in respect to that of **6** for the absence of the carbonyl of carboamide group and for the significant upfield shift (Δδ 7.3) of C-2