SHORT REPORT

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Bioactive Chemical Constituents of a Sterile Endophytic Fungus from *Meliotus dentatus*

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Abstract: Chemical and biological investigations of the endophytic fungus of an unidentified Ascomycete, isolated from *Meliotus dentatus* led to the isolation of six known polyketide metabolites (**1–6**) and two steroids (**7** and **8**). Compounds **1–3,** and **5** were tested for antibacterial, antialgal and antifungal activities. Compounds **2**, **3**, and **5** showed good activity against the alga *Chlorella fusca,* while compounds **2** and **3** were active against the Gram negative bacterium *Escherichia coli* and compounds **3** and **5** also against the Gram positive *Bacillus megaterium*. Similarly, compounds **3** and **5** also showed antifungal activity against *Microbotryum violaceum.*

***Keywords*:** Endophytic fungus; *Meliotus dentatus*; polyketides; steroids; biological activity

1. Fungal Source

Endophytic fungi are a rich source of novel organic compounds with interesting biological activities and a high level of biodiversity. They represent a relatively unexplored ecological source, and their secondary metabolism is particularly active because of their metabolic interactions with their hosts [1]. Within our screening program for fungicidal, herbicidal and antibacterial fungal secondary metabolites, we investigated an endophytic fungus, an unidentified Ascomycete that had been isolated from *Meliotus dentatus,* for chemical and biological investigations. The fungus was cultivated for 27 days on solid medium and the culture was extracted with ethyl acetate. The endophytic fungus was deposited in the culture collection of the Institute of Microbiology, Technical University of Braunschweig, Germany (TUBS 6650).

2. Previous Studies

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There have been no previous studies on the metabolites of this fungal isolate.

3. Present Study

The fungal strain 6650, isolated following surface sterilization from the plant *Meliotus dentatus* from the coastal area of the Baltic Sea, Ahrenshoop, Germany, was cultivated on 10 L of 5 % w/v biomalt solid agar medium at room temperature for 27 days [2]. The cultures were then extracted with ethyl acetate to afford 6.5 g of residue after removal of the solvent under reduced pressure. The extract was separated into three fractions by column chromatography (CC) on silica gel, using a gradient of *n*-hexane-ethyl acetate (90:10, 50:50, 0:100). Fraction B was further separated by silica gel column chromatography eluting with *n*-hexane-ethyl acetate (2:8) to give 7-hydroxyphthalide (**1**, 20 mg) and 4-hydroxyphthalide (**2**, 5.5 mg). Fraction C was eluted with a mixture of *n*-hexane-ethyl acetate (2.5:7.5), yielding 5-methoxy-7-hydroxyphthalide (**3**, 4.2 mg) and 5,7-dihydroxyphthalide (**4**, 4.6 mg), while fraction D [*n*-hexane-ethyl acetate (7:3)] subjected to CC, afforded (3*R*,4*R*)-*cis*-4-hydroxymellein (**5**, 7.5 mg) and (3*R*,4*R*)-*cis*-4-hydroxy-5-methylmellein (**6**, 3.0 mg). Similarly, fraction A was separated by CC on silica gel with *n*-hexane-ethyl acetate (8.5:1.5) to give ergosterol (**7**, 13.5 mg) and 5α,8α-epidioxyergosterol (**8**, 6.5 mg).

Bioactivity Tests: The tested compounds were dissolved in acetone at a concentration of 1 mg/mL. 50 µL of the solution were pipetted onto a sterile filter disc, which was placed onto an appropriate agar growth medium for the respective test organism and subsequently sprayed with a suspension of the test organism on the appropriate medium (MPY or NB) [3]. The test organisms were *Escherichia coli* (NB), *Bacillus megaterium* (NB), *Microbotryum violaceum* (MPY) and *Chlorella fusca* (MPY). The radius of zone of inhibition was measured in mm.

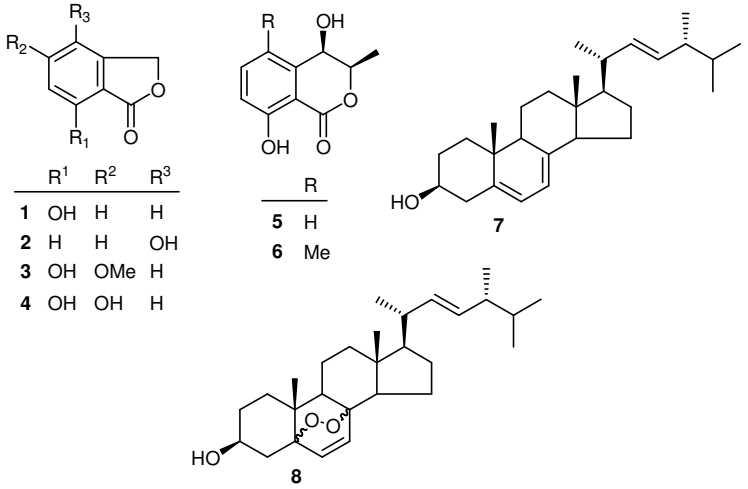

Figure 1. Compounds **1–8** isolated from endophytic fungus *Meliotus dentatus*

Antibacterial, antialgal, and antifungal activities of compounds **1–3**, and **5** were determined according to Höller et al. [3] (Table 1). Compounds **2**, **3**, and **5** showed good activity against the alga *Chlorella fusca,* while compounds **2** and **3** were active against the Gram negative bacterium *Escherichia coli* and compounds **3** and **5** also against the Gram positive *Bacillus megaterium.* Similarly, compounds **3** and **5** also showed antifungal activity against *Microbotryum violaceum.* Compound **1** was inactive in these tests.

Table 1: Biological activity of compounds **2**, **3** and **5** in an agar diffusion test

Compound	antialgal	antifungal	antibacterial	antibacterial
	Chl [a]	Mb	Bm	Ec
2	gi 7	0	0	gi 6
3	gi 10	gi 7	gi 7	gi 8
5	gi 9	gi 8	gi 6	0

[a] *Chlorella fusca* (Chl), *Microbotryum violaceum* (Mb), *Escherichia coli* (Ec), and *Bacillus megaterium* (Bm). Application of pure substances at a concentration of 0.05 mg (50 µL of 1 mg/mL). The radius of zone of inhibition was measured in mm.
gi: indicates that some growth occurred in the zone of inhibition

The antifungal activity of compounds **3** and **5** against the phytopathogenic fungus *Microbotryum violaceum* suggests that the endophytic fungus in *Meliotus dentatus* could protect the host by producing metabolites, which may be toxic or even lethal to phytopathogens and highlights the potential of endophytic fungi in producing bioactive metabolites [12, 13, 14].

The EtOAc extract of an endophytic fungus, an unidentified Ascomycete which had been isolated from *Meliotus dentatus,* was selected for chemical and biological investigations. The extract was chromatographed on silica gel to give four pthalides (**1–4**), two isocoumarin (**5**,**6**) and two steroids (**7**,**8**) (Figure 1). These eight compounds were identified as 7-hydroxyphthalide (**1**) [4], 4-hydroxyphthalide (**2**) **[**5**]**, 5-methoxy-7-hydroxyphthalide (**3**) [6], 5,7-dihydroxyphthalide (**4**) [7], (3*R*,4*R*)-*cis*-4-hydroxymellein (**5**) [8], (3*R*,4*R*)-*cis*-4-hydroxy-5-methylmellein (**6**) [9], ergosterol (**7**) [10], and 5α,8α-epidioxyergosterol (**8**) [11], by comparison of ^{1}H, ^{13}C NMR (Bruker 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR), and EIMS (VG 7070 mass spectrometer operating at 70 eV) data with reported data.

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