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Activity of the pterophyllins 2 and 4 against postharvest fruit pathogenic fungi. Comparison with a synthetic analog and related intermediates

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ABSTRACT

The antifungal activity of pterophyllin 2, pterophyllin 4, a 5-desmethyl analog of the latter and some of their synthetic intermediates, against three postharvest phytopathogenic fungi, was evaluated. The target fungi were Rhizopus stolonifer, Botrytis cinerea and *Monilinia fructicola*, which affect fruits worldwide, causing important economic losses. The tests were carried out with imazalil and carbendazim as positive controls. Minimum inhibitory concentrations and minimum fungicidal concentrations were determined, and the morphology of the colonies was examined microscopically. In liquid medium, it was found that pterophyllin 4 exhibited selective fungicidal activity toward *M. fructicola*, whereas its congener pterophyllin 2 proved to be less potent and not selective and the 5-desmethyl analog of pterophyllin 4 displayed a different activity profile. Morphological changes were observed in the colonies exposed to pterophyllin 4. The results highlighted the importance of small structural features for the antifungal behavior and also suggested that, in Nature, the pterophyllins may act as plant defenses against pathogens.

1. Introduction

Plant diseases caused by phytopathogenic fungi are responsible for important economic losses, which arise mainly from crop yield reduction, but are also a result of diminished quality and safety of the products. Sometimes, they also represent a risk for human and animal health, due to food contamination [1].

Rhizopus stolonifer (Ehrenb.: Fr.) Vuill, *Botrytis cinerea* (Pers.: Fr.) and *Monilinia fructicola* (G. Wint.) Honey are widely known phytopathogens and three of the main pathogenic fungi of concern to Argentine exports of fruits and also to their producers.

R. stolonifer is one of the most common and fastest-growing phytopathogenic species, especially under mild moisture conditions, being considered as one of the most devastating threats [2a]. It attacks a wide variety of hosts, causing the black mold rot; its quick penetration and colonization abilities result in fast spreading from infected to healthy fruit, at any stage between processing and consumers' houses.

B. cinerea is a ubiquitous and destructive plant pathogen, responsible for the botrytis bunch rot or gray mold, which causes damage on a large number of economically important agricultural and horticultural crops. It is an important disease which produces heavy losses to table and wine grapes [2b]. This pathogen is currently being controlled with pre- and postharvest fungicides [2c].

On the other hand, *M. fructicola* is the causal agent of the brown rot, a serious disease that affects the quality of peaches from the blossom period up to the harvest and storage stages. Its broad dissemination results in heavy production losses. For the Chinese market, the damage has been estimated as high as over 20% [2d].

Physical methods (X-rays and radio frequency, cold/hot water) [3], some inorganic salts [4], and synthetic biocides including sanitizing products [5] are among the main alternative strategies employed to ameliorate the threat posed by phytopathogens. These approaches are also being complemented by emerging, non-conventional resources, such as the use of natural antimicrobials [6] as biochemical control agents, and antagonist microorganisms [7] as biological control means.

All of them have some drawbacks, which range from damage to the sensory quality of the fruits, including their firmness [8a,b], to limited or variable efficacy [8c]. Further, the biological control agent *Saccharomyces cerevisiae* has been reported as the causal agent of some clinical infections [9], especially dangerous in immunocompromised patients [10].

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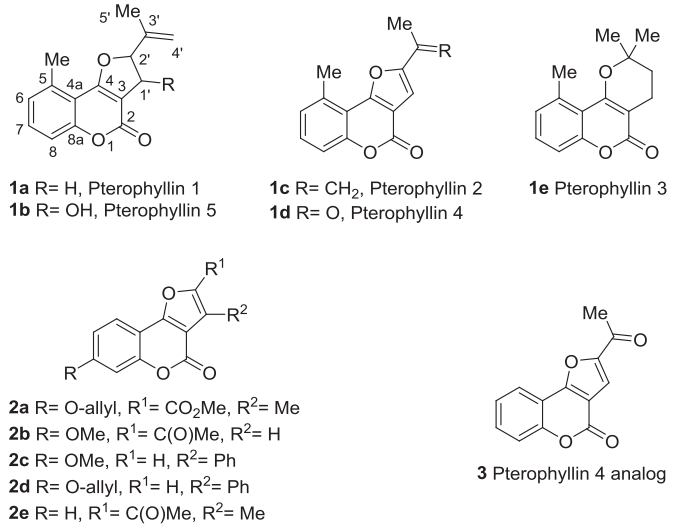


Fig. 1. Chemical structures of the pterophyllins 1–5 (**1a–e**), osthole derivatives (**2a–d**) and a pterophyllin 4 analog (**3**).

Since regulations on the use of new and existing fungicides are becoming more and more stringent, it urges to identify and develop new chemical entities with antifungal properties. However, increasing consumer awareness [11a,b], coupled to concerns regarding food quality and safety, demand from the novel fungicides an increasing eco-friendly character, as well as lack of cross-resistance with existing products [11c].

Naturally-occurring non-toxic chemicals have emerged as promising alternatives to the synthetic fungicides, as they may furnish effective protection against postharvest deterioration without exhibiting unwanted effects. Different natural products [12], semisynthetic compounds [13a,b], and plant derivatives [13c], including extracts, formulations based on chitosan [14], essential oils [15a,b], polyphenolics [15c,d], and carnauba wax [15e] have been tested as part of this strategy.

The pterophyllins 1–5 (**1a–e**) are 5-methyl substituted coumarin derivatives (Fig. 1), isolated in tiny amounts from *Ekebergia pterophylla* (C.D.C.) Hofmeyr (Meliaceae), a small evergreen tree known as Rock Ash, which grows on the Natal Group Sandstone outcrops, in South Africa [16]. Except for pterophyllin 3 (**1e**), they share a furo[3,2-*c*] coumarin core. Pterophyllin 2 (**1c**) was isolated from the methanolic extract of the bark (4.1 mg, 0.0016%), whereas pterophyllin 4 (**1d**) was found in the chloroformic extract of the wood (4.6 mg, 0.00077%).

To date, the bioactivity profile of the pterophyllins remains unknown. It has been shown that some furo[3,2-*c*] coumarin derivatives of osthole (7-methoxy-8-prenyl coumarin), such as **2a–e**, are active against phytopathogenic fungi [17]. Since **2e** can be regarded as an analog of pterophyllin 4 (**1d**), we conjectured that the pterophyllins may display antifungal activity. The small amounts of the natural products found in Nature and the difficulty of preparing analogs from them suggested chemical synthesis as a convenient strategy for their study.

We have recently reported the total syntheses of pterophyllin 2 (**1c**) and pterophyllin 4 (**1d**) [18a]. Therefore, herein we wish to report the study of the antifungal activity of **1c**, **1d**, and **3** (a 5-desmethyl analog of **1d**) against *R. stolonifer*, *B. cinerea* and *M. fructicola*, three relevant phytopathogenic fungi, that infect fruits mainly during the postharvest stage. For the sake of comparison, some heterocyclic synthetic intermediates toward the pterophyllins [18a] have also been included.

2. Results and discussion

2.1. Chemistry

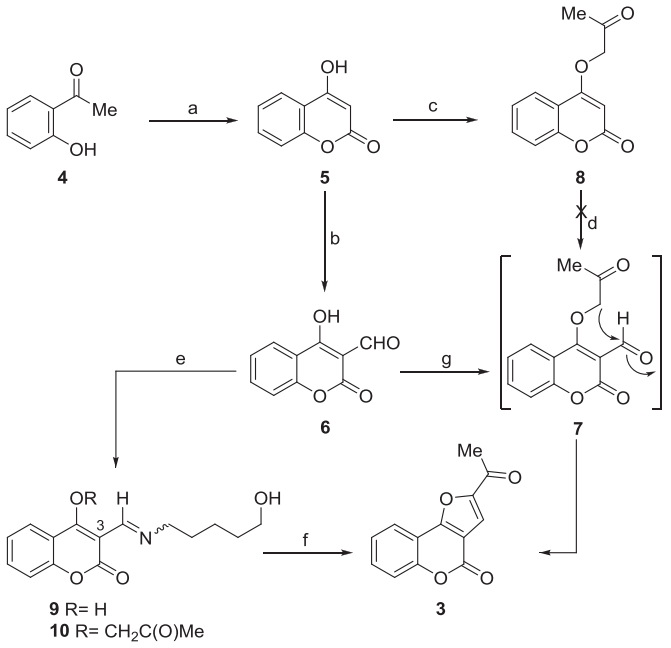
The access to the proposed analog **3** was achieved through a strategy involving the scarcely precedented [18a] Casnati-Skattebøl C-3 selective formylation of a 4-hydroxycoumarin and a one-pot acetonylation-cyclodehydration sequence, as its main features.

To that end, the economic and readily available 2-hydroxy acetophenone (**4**) was submitted to reaction with diethyl carbonate in THF; when K^{t}BuO was employed as basic promoter, the reaction gave 82% yield of the expected 4-hydroxy coumarin intermediate **5** [19a]. In turn, the latter was exposed to anhydrous paraformaldehyde in THF at 50 °C, in the presence of the $MgCl_2$-Et_3N reagent system [18b,c], cleanly affording 91% yield of the 3-formyl derivative **6** [19b]. The use of carefully dried reagents and anhydrous solvent in the Casnati-Skattebøl reaction was critical for the attainment of good yields. It was observed that the transformation failed, or its performance was sensitively lower when commercial paraformaldehyde (> 5% H_2O) was employed or when the $MgCl_2$ was not scrupulously dry.

Attempts at *O*-acetonylation of **6** under different base-promoted conditions (K_2CO_3, $MgCO_3$) failed to afford the expected intermediate **7**, and resulted in degradation of the starting material. Therefore, in search of an alternative path, the *O*-acetonylation of **5** was performed instead, with chloroacetone and K_2CO_3 in absolute EtOH. The reaction furnished 52% of the acetonyl ether **8**; [20] however, the latter missed to deliver the 3-formyl derivative **7**, when the Vilsmeier-Haack conditions ($POCl_3$, DMF) were employed.

Suspecting that the high reactivity of the formyl moiety of **6** was responsible for the observed results, the aldehyde was submitted to imination with 5-aminopentanol under azeotropic conditions [21], to afford **9** in 75% yield. This was followed by the one-pot K_2CO_3-assisted *O*-acetonylation of the so formed imine **9** in DMF and the subsequent cyclization to **3** through the acetonyl ether **10**. Fortunately, the latter stages resulted in direct access to the tricycle **3**, albeit in a meager 28% yield from **9** (4 steps, 16% overall yield from **4**) (Scheme 1).

In order to simplify and improve the performance of the synthetic sequence, the use of different bases was explored. Luckily, stirring a warm dichloromethane mixture of **6** and chloroacetone with activated basic alumina (Brockmann I) smoothly furnished the final product **3** in 88% yield, presumably through the intermediacy of the acetonyl ether



Scheme 1. *Reagents and conditions*: a) 1. K^{t}BuO, THF, r.t., 10 min.; 2. Et_2CO_3, reflux, 12 h (82%); b) $MgCl_2$ (anh.), Et_3N, $(CH_2O)_n$, THF, 50 °C, 1 h (91%); c) $ClCH_2COCH_3$, K_2CO_3, EtOH, 45 °C, overnight (52%); d) $POCl_3$, DMF, 0 °C; e) $H_2N(CH_2)_4CH_2OH$, PhMe, 90 °C, 1 h (75%); f) $ClCH_2COCH_3$, K_2CO_3, DMF, 75 °C, overnight (28%); g) $ClCH_2COCH_3$, Al_2O_3, CH_2Cl_2, 40 °C, 72 h (**3**, 88%).