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Research article

Phytotoxicity of umbelliferone and its analogs: Structure–activity relationships and action mechanisms

Le Pan [a, b], Xiu-zhuang Li [a], Zhi-qiang Yan [a], Hong-ru Guo [a, b], Bo Qin [a, *]

[a] Key Laboratory of Chemistry of Northwestern Plant Resources of CAS and Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China

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ABSTRACT

Two coumarins, umbelliferone and daphnoretin, were isolated from roots of *Stellera chamaejasme* L; the former had been identified as one of the main allelochemicals in our previous studies. Both of them have the skeleton of 7-hydroxycoumarin, but showed different phytotoxic effects. Umbelliferone and its analogs were then prepared to investigate the structure–activity relationship of hydroxycoumarins and screened for phytotoxicity. The inhibitory effects varied observably in response to the coumarin derivatives, especially umbelliferone (**1**), 7-hydroxy-4-methylcoumarin (**3**) and coumarin (**10**) displayed strong inhibition of lettuce and two field weeds, *Setaria viridis and Amaranthus retroflexus*, and compounds **11** and **12** also exhibited phytotoxic activity with species specificity. The number and location of hydroxyl groups were importantly responsible for the phytotoxicity. A C7 hydroxyl group was considered to be a potentially active site and methyl substitution at the C4 position contributed significantly to the activity. The phytotoxic mechanism was briefly studied with umbelliferone by evaluating the reactive oxygen species (ROS) and chlorophylls level in lettuce seedlings. The results showed that umbelliferone induced the accumulation of ROS in the root tip and significantly decreased the chlorophyll content in the leaves. Thus, a ROS-mediated regulation pathway and the inhibition of photosynthesis were definitely involved in the phytotoxicity of umbelliferone.

1. Introduction

Allelopathy plays an important role in ecological adaptation of plants by causing positive or negative effects on their surrounding organisms. Biochemicals, released from the plants or their residues, are known as allelochemicals, which are regarded as the basis of allelopathic events (Babula et al., 2009). Allelochemicals, especially those involving causing damage to receptor plants, have drawn increasing attention in the development of promising alternatives to conventional herbicides in crop protection (Nebo et al., 2014). Coumarins are phenolic metabolites commonly found in many plant species with diverse bioactivities, including anticoagulant (Maitland-van der Zee et al., 2014), antibacterial (Yasunaka et al., 2005), antiviral (Shokoohinia et al., 2014) and anticancer (Li et al., 2013). In plants, coumarins have been reported to provide defense against pathogens, response to abiotic stresses, regulation of oxidative stress and probably hormonal regulation (Bourgaud et al., 2006). Coumarins or the aqueous extracts from their source plants inhibit the germination and growth of plants (Haig et al., 2009), and some of them have been determined to be allelochemicals (Itani et al., 2013; Kalinova et al., 2007).

Umbelliferone is an important naturally occurred coumarin distributed in many plants (Li et al., 2011; Silva et al., 2014). In our earlier studies, umbelliferone was isolated together with daphnoretin and some flavonoids from the roots of *Stellera chamaejasme* L, a widespread toxic weed in the dry regions of the western Himalayas and southwestern China (Cui et al., 2014; Yan et al., 2014). Furthermore, umbelliferone was found in the root zone soils of *S. chamaejasme* (Guo et al., 2015). By showing inhibitory effects against *Festuca rubra* L. and *Medicago sativa* seedlings, umbelliferone was recognized as one of the important allelochemicals, proposed to be responsible for the devastating spread of *S. chamaejasme* in grass land (Guo et al., 2015). Both

* Corresponding author. Key Laboratory of Chemistry of Northwestern Plant Resources and Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, 18# Tianshuizhong Rd., Lanzhou 730000, China.
E-mail address: bqin@licp.cas.cn (B. Qin).

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umbelliferone and daphnoretin have the skeleton of 7-hydroxycoumarins (Fig. 1), but the latter was formed by substituting C7 hydroxyl group with 6-methoxy-7-hydroxycoumarins, and as a result, the phytotoxicity was significantly decreased. Therefore, we have focused research on the activity contribution of the C7 hydroxyl group in this group of metabolites.

A series of hydroxycoumarin analogs were used to investigate the relationships between their structures and phytotoxic activities on standard target plants: lettuce (Nebo et al., 2014) and two common field weeds, *Setaria viridis and Amaranthus retroflexus*. The effects on ROS production and chlorophylls level were also assayed to reveal the mechanisms of umbelliferone phytotoxicity.

2. Materials and methods

2.1. Chemicals

All reagents were of A.R. grade and used with no purification. 4, 7-dihydroxycoumarin (**2**), 6-methoxy-4-methycoumarin (**8**) and 7-amino-4-methycoumarin (**11**) were synthesized according to previous procedures with minor modification (Bulut and Erk, 1996; Shen et al., 2010; Naik et al., 2007) and shown in Scheme 1. Their structures were characterized with ^{1}H and ^{13}C NMR performed on a Bruker AM-400BB instrument (Bruker, Karlsruhe, Germany) with TMS as internal standard, operating at 400 MHz. The chemical shift values are on δ scale and the coupling constant values (*J*) are in Hertz. The other hydroxycoumarin analogs were obtained from J&K Chemical. All compounds tested for activity had a purity of >95% and the structures were shown in Fig. 2.

2.1.1. 4, 7-Dihydroxycoumarin (2)

A mixture of resorcinol (0.10 mol, 11.01 g), malonic acid (0.10 mol, 13.40 g), anhydrous zinc chloride (0.32 mol, 43.62 g) and phosphorus oxychloride (33 mL) was heated with stirring at 65 °C for 12 h. The mixture was cooled and quenched with ice-water. The crude product was collected and dissolved in 5% sodium carbonate. An oily byproduct was removed and acidification of the remaining solution gave the residues which were recrystallized from ethyl acetate to yield the product. It was obtained as yellow solid (62%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 5.55 (s, 1H), 6.68 (s, 1H), 6.76 (d, 1H), 7.63 (d, 1H), 10.54 (s, 1H), 12.27 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 87.88, 102.03, 107.76, 112.61, 124.61, 155.52, 161.77, 162.51, 166.27.

2.1.2. 6-Methoxy-4-methycoumarin (8)

80% sulfuric acid (30 mL) was added drop wise to a mixture of 4-methoxyphenol (0.02 mol, 2.48 g) and ethyl acetoacetate (0.02 mol, 2.6 g). The mixture was heated with stirring at 100 °C for 5 h. The mixture was cooled and quenched with ice-water. The solid product was separated, filtered out dried, and the crude product was recrystallized from ethanol. It was obtained as white solid (75%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.43 (s, 3H), 3.84 (s, 3H), 6.38 (s, 1H), 7.18 (s, 1H), 7.19 (d, 1H, $J = 9.2$ Hz), 7.31 (d, 1H, $J = 9.2$ Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 18.16, 55.74, 108.12, 114.68, 117.49, 120.10, 147.20, 152.99, 155.58, 159.89.

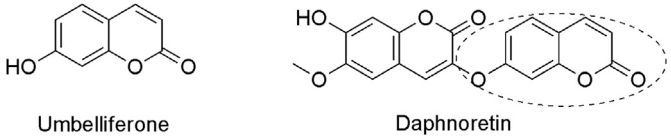
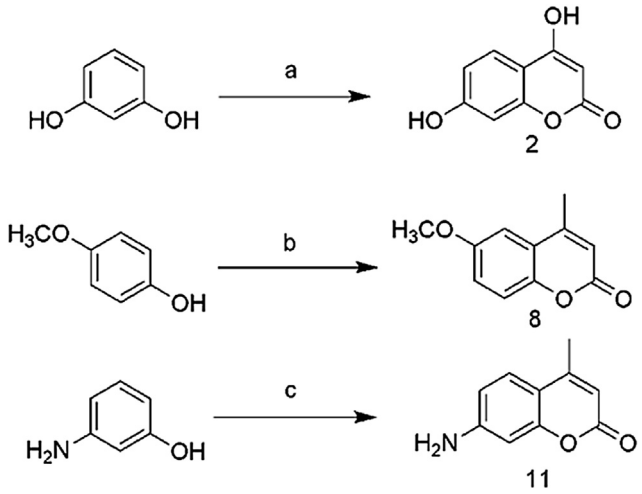


Fig. 1. The structures of umbelliferone and daphnoretin.



Scheme 1. The synthesis of compounds **2**, **8** and **11**. Reagents and conditions: (a) malonic acid, anhydrous $ZnCl_2$, $POCl_3$, 65 °C; (b) ethyl acetoacetate, 80% H_2SO_4, 100 °C; (c) ethyl acetoacetate, $ZnCl_2$, 80 °C.

2.1.3. 7-Amino-4-methycoumarin (11)

A mixture of m-aminophenol (0.09 mol, 9.81 g), ethyl acetoacetate (0.1 mmol, 13.01 g) and zinc chloride (0.01 mol, 1.36 g) was heated with stirring at 80 °C for 10 h. The mixture was cooled to room temperature and quenched with ice water. The solid product was separated, filtered out, dried, and the crude product was recrystallized from ethanol. It was obtained as a pale yellow solid (65%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.36 (s, 3H), 6.03 (s, 1H), 6.57 (d, 1H, $J = 7.2$ Hz), 7.26 (d, 1H), 7.36 (d, 1H, $J = 8.8$ Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 18.53, 101.17, 110.29, 111.64, 125.79, 142.77, 147.71, 150.99, 153.23, 160.64.

2.2. Bioassay

Lactuca sativa (lettuce) and two common field weeds in China, *S. viridis and A. retroflexus*, were selected as targets (a monocotyledonous plant and a dicotyledonous plant) to screen the phytotoxicity of coumarin analogs. Seeds were washed with distilled water five times, and germinated in moist dishes at 25 °C in dark. After 48 h, the seedlings were transferred into 6-well plates with three seedlings per well and three wells in each treated group. The seedlings were treated with solutions of coumarin analogs at 100 μg/mL (0.5% DMSO), with 0.5% (v/v) DMSO and distilled water as negative control. Then the plates were sealed with parafilm and incubated at 25 °C in dark for 48 h. After treatments, the shoot and root length of all plants were measured and compared to those of controls (Liu et al., 2013; Hiradate et al., 2004). The phytotoxicity of umbelliferone on lettuce was further evaluated at lower concentrations to determine concentration–activity relationships.

2.3. Chlorophyll content of lettuce leaves

The chlorophyll content was determined by a previous procedure with minor modifications (Sree et al., 2015; Yan et al., 2015). The two-day-old seedlings were treated with the hydroxycoumarins under a 16 h/8 h day/night photoperiod at 25 °C for 48 h, the fresh lettuce leaves (50 mg) were collected and homogenized in 4.0 mL of 80% acetone and centrifuged at 2000 *g* for 5 min. The absorbance of the supernatant was measured at 663.2 nm and 645.8 nm and the results were recorded. The contents of chl *a* and *b* were calculated according to Wellburn (1994).