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# Cultured roots of *Xiphidium caeruleum*: Phenylphenalenones and their biosynthetic and extractant-dependent conversion

Yu Chen [a, b], Christian Paetz [a], Riya C. Menezes [a], Bernd Schneider [a, *]

[a] Max-Planck Institut für Chemische Ökologie, Beutenberg Campus, Hans-Knöll-Strasse 8, 07745, Jena, Germany
[b] Jiangsu Key Laboratory for Research and Utilization of Plant Resources, Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, Qianhu Houcun 1, 210014, Nanjing, China

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ABSTRACT

Phytochemical investigation of root cultures of *Xiphidium caeruleum* (Haemodoraceae) resulted in the structure elucidation of five previously undescribed phenylphenalenone-type compounds, structure revision of a phenylphenalenone glucoside, and identification of nine additional constituents previously reported from other Haemodoraceae and Musaceae plants. The observed extractant-dependent metabolic profiles indicated that phenylphenalenones had been converted hydrolytically and oxidatively. Stable isotope labeling experiments extended the understanding of the phenylphenalenone pathway in plants and provided evidence for a network of biosynthetic and spontaneous conversions linking phenylphenalenones and their derivatives detected in extracts of cultured roots of this plant.



## 1. Introduction

Previous phytochemical studies of different Musaceae and Haemodoraceae plants revealed the family- or species-specific occurrence of distinct structural types of phenylphenalenones. 4-Phenylphenalenones, for example, are characteristic of the Musaceae and have not been reported from the Haemodoraceae, and 1,2,5,6-tetraoxygenated phenylphenalenones are known mostly from the Haemodoroideae subfamily of the Haemodoraceae. Moreover, the organ- and cell-specific distribution of special phenylphenalenone derivatives, e.g. of oxa- and aza-phenylphenalenones, has been reported (Chen et al., 2016; Opitz and Schneider, 2002; Opitz et al., 2003). Species-specific differences in biosynthetic pathway branches are one of the reasons for the structural diversity of phenylphenalenones. The diversity of chemical structures implies a functional diversity in the ecological interaction of the phenylphenalenone-producing plants with pathogenic organisms and therefore merits further phytochemical, biosynthetic and bioactivity studies.

*Xiphidium caeruleum* Aubl. is one of the plants with the largest diversity of phenylphenalenones. Forty-five phenylphenalenones have been reported to date from different plant organs and from root cultures of *X. caeruleum* (Chen et al., 2016; Cremona and Edwards, 1974; Opitz et al., 2002; Fang et al., 2012a). Preliminary LC-NMR studies have also shown that phenylphenalenone derivatives are the major specialised metabolites found in root cultures of *X. caeruleum* (Schneider et al., 2005). Since phenolics are known to undergo *O*-methylation when extracted with methanol and a case of spontaneous formation of *O*-methyl derivatives of phenylphenalenones has been recently reported (Otálvaro et al., 2010), acetone and acetonitrile, in addition to methanol, were used to re-investigate the phytochemical profile of *X. caeruleum* root cultures. The metabolic profiles we found prompted us to ask whether the extraction capacity of these solvents is responsible for the appearance of different metabolites in the extract and if the structure of the metabolites is modified by reactions with the solvent. Here we also report the use of stable isotopic labeling and NMR analysis to observe the formation of different phenylphenalenone-type compounds, either by biosynthetic or spontaneous reactions.

* Corresponding author.
*E-mail address:* schneider@ice.mpg.de (B. Schneider).

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## 2. Results and discussion

### 2.1. Phytochemical profiles of root cultures obtained by using different solvents for extraction

The phenylphenalenones obtained from root cultures of *X. caeruleum* differ considerably in their polarity, and were extracted from the plant material using different solvents and extraction procedures. Identical amounts of lyophilized root material were extracted using three different solvents: 80% acetone-water, 80% acetonitrile-water and methanol. The extracts were evaporated to dryness, reconstituted in identical volumes of the previous extractant, and subjected to high-performance liquid chromatography–photodiode array detection–electrospray mass spectrometry (HPLC-PDA-ESIMS) analysis. Significant differences were observed in the phytochemical profiles of different root culture extracts (Fig. 1A–C). The intensity of peaks indicated that methanol was the most efficient solvent for glucosides **1**–**6**, while 80% acetone-water and 80% acetonitrile-water resulted in lower levels of glucosides but higher levels of phenylphenalenone aglycones **7**–**15**. Compounds **1**–**6** are not produced by spontaneous reaction with the solvent but apparently are natural products because they were detected in all three extracts. Furthermore, arteficial *O*-methylation by using methanol as an extractant did not occur because no additional peak was detected in the HPLC profile C compared to traces A and B (Fig. 1). Compounds **7**, **8**, and **9a** occurred only in the 80% acetone-water extract, while compounds **11** and **12** were found exclusively in the 80% acetonitrile-water extract. To understand the remarkably different effects of these three extraction procedures, the structures of chemical constituents were elucidated.

### 2.2. Structure elucidation of phenylphenalenones from in vitro root cultures

In total, 15 phenylphenalenones including six phenylphenalenone glucosides were identified from *in vitro* root cultures of *X. caeruleum*. Among them, compounds **6** to **10** are described here for the first time (Fig. 1D), and compound **5** has been revised from a previously reported structure. Compound **5** was isolated as an orange powder. The molecular formula was established to be $C_{29}H_{26}O_{11}$ by its high-resolution electrospray ionization mass spectrum (HRESIMS) for the $[M+H]^+$ at *m/z* 551.1530 (calcd for $C_{29}H_{27}O_{11}$, *m/z* 551.1553). The electrospray ionization mass spectrum (ESIMS) data showed a molecular ion peak $[M+H]^+$ at *m/z* 551 and a strong fragment peak at *m/z* 303 as a base peak. The aromatic part of the $^1$H NMR spectrum of **5** (Table 1) showed four doublets of two AB spin systems at δ 7.63 (1H, *J* = 9.1 Hz, H-5), 8.09 (1H, *J* = 9.1 Hz, H-6), 8.27 (1H, *J* = 8.3 Hz, H-7) and 7.49 (1H, *J* = 8.3 Hz, H-8), a singlet of an aromatic proton at δ 7.69 (1H, s, H-3), and signals

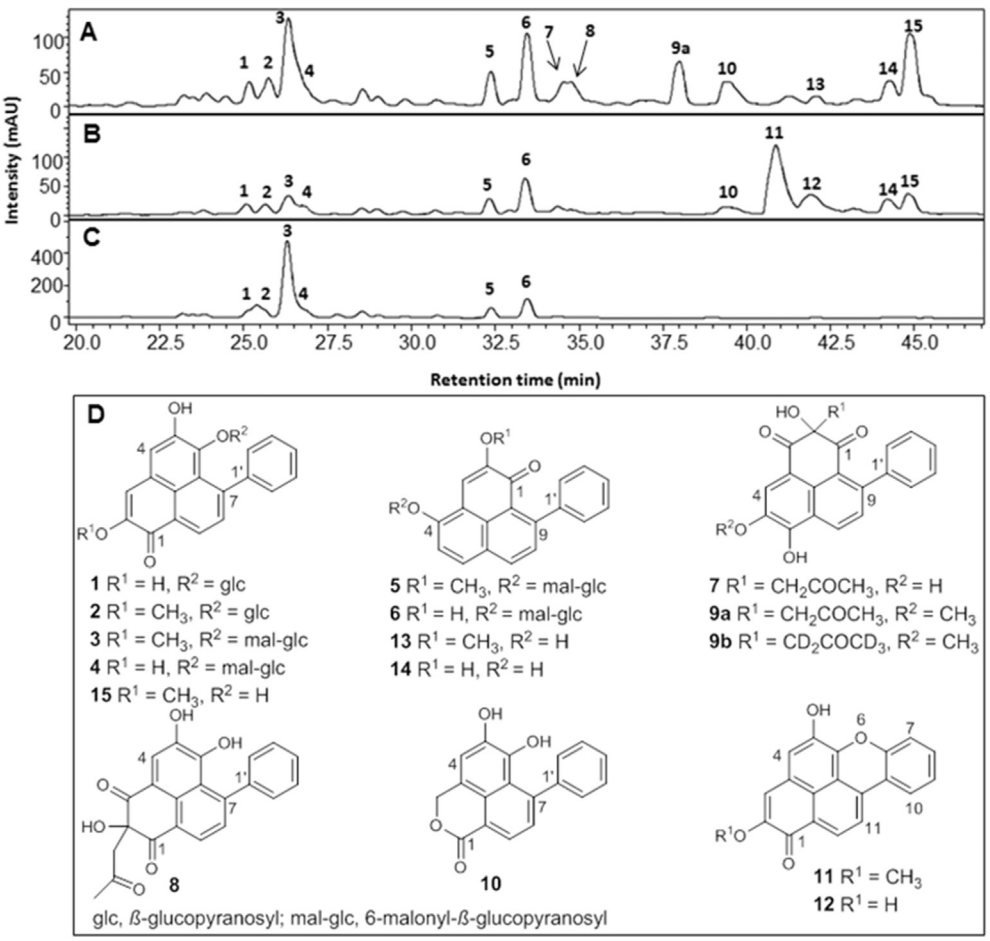


**Fig. 1.** HPLC profile (λ = 254 nm) of different extracts of root cultures of *Xiphidium caeruleum*. Equal amounts (30 mg) of dried plant material were used in each case to produce 100 μL of the extracts. A) Extract obtained by homogenizing root cultures in 80% acetone-water and shaking for 24 h. B) Extract obtained by homogenizing root cultures in 80% acetonitrile-water and shaking for 24 h. C) Extract obtained by homogenizing root cultures in methanol for 3 × 3 min. D) Structures of compounds **1** to **15** isolated from root cultures of *Xiphidium caeruleum*. Compound **9b** was obtained from incubating a suspension of root cultures with acetone-$d_6$ (see Experimental Section).

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