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Organ-specific analysis of phenylphenalenone-related compounds in *Xiphidium caeruleum*

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Dedicated to Professor Günter Adam on the occasion of this 70th birthday

Abstract

The distribution pattern of phenylphenalenone-type compounds was investigated in vegetative and reproductive organs of *Xiphidium caeruleum*. The highest total molar concentration, up to 30 μmol g^{-1} fr. wt, was detected in the root tip and the stamen. Accumulation of specific phenylphenalenone-related metabolites including glycosides was found in the hypogeal plant parts, the leaves, and the reproductive organs of the inflorescence. Putative biosynthetic relationships and the role of these compounds in plant defence are discussed.

Keywords: *Xiphidium caeruleum*; Haemodoraceae; Accumulation; Distribution; Organ-specificity; Phenylphenalenones

1. Introduction

Phenylphenalenones and related compounds are known from the monocotyledonous plant families Haemodoraceae (Cooke and Edwards, 1981), Pontederiaceae (Greca et al., 1992), Musaceae (Luis et al., 1995; Kamo et al., 1998), and Strelitziaceae (Hölscher and Schneider, 2000). Due to their intense red or orange color, phenylphenalenones are visually detectable in pigmented roots and rhizomes, which seem to be the richest source of these natural products (Cooke, 1970; Edwards and Weiss, 1974; Luis et al., 1996; Hölscher and Schneider, 1999). Sterile root cultures proved as system to isolate new compounds of that type as well (Hölscher and Schneider, 1997). In addition to hypogeal parts, phenalenone-type compounds were also found in other plant materials such as leaf-bases (Bick and Blackman, 1973), infected leaves (Luis et al., 1993), flowers (Edwards and Weiss, 1972; Bazan and Edwards, 1976), and fruits (Luis et al., 1993; Kamo et al., 1998) including fruit capsules (Edwards and Weiss, 1970).

Studies on several *Musa* varieties showed that phenylphenalenone levels are higher in the roots than in the leaves (Otalvaro et al., 2002). However, there is no detailed information on the organ-specific distribution of these natural products within any plant and no proportions of various compounds within specific plant organs have been quoted. Based on recent phytochemical studies (Opitz et al., 2002), in the present paper investigations on the distribution pattern of some representative phenylphenalenones and related compounds in intact plants of *Xiphidium caeruleum* Aubl. (Haemodoraceae) are reported. Beside the comparison of the distribution between the plant organs, particular attention has been paid to differences between various parts of the leaves. This investigation is of interest with respect to the function of phenylphenalenones in plants.

2. Results

2.1. Overall distribution in vegetative organs

The distribution pattern of phenylphenalenone-type natural products **1**–**11** (Fig. 1) of *Xiphidium caeruleum* was studied in the shoot and root system. Fig. 2a shows the relative abundance of compounds **1**–**11** in representative segments of leaves, stems, mature roots and root tips. Although there were minor but significant differences between the proportions of compounds **1** and **3** in the leaves and the stem, the overall pattern was almost similar in both organs. Interestingly, there were striking differences between the aerial and hypogeal plant parts. The glucoside **7** accounted for approximately

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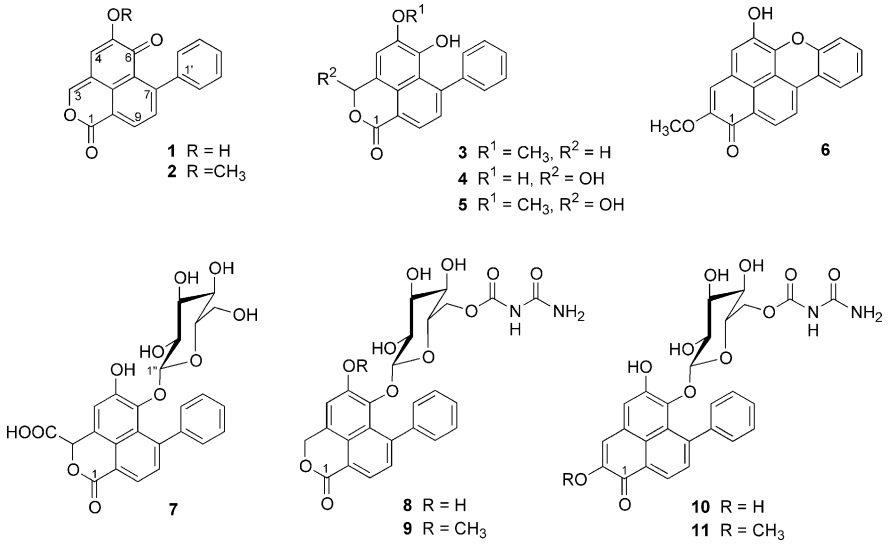

Fig. 1. Selected phenylphenalenone-type compounds from *Xiphidium caeruleum*: 5-hydroxy-7-phenylbenzo[*de*]isochromene-1,6-dione (lachnanthopyrone) (**1**), 5-methoxy-7-phenylbenzo[*de*]isochromene-1,6-dione (**2**), 6-hydroxy-5-methoxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (**3**), 3,5,6-trihydroxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (**4**), 3,6-dihydroxy-5-methoxy-7-phenyl-3*H*-benzo[*de*]isochromene-1-one (**5**), 5-hydroxy-2-methoxy-6-oxa-benzo[*def*]chrysen-1-one (**6**), 3-carboxy-5-hydroxy-6-*O*-β-D-glucopyranosyl-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (**7**), 6-*O*-[(6″-*O*-allophanyl)-β-D-glucopyranosyl]-5-hydroxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (**8**), 6-*O*-[(6″-*O*-allophanyl)-β-D-glucopyranosyl]-5-methoxy-7-phenyl-3*H*-benzo[*de*]isochromen-1-one (**9**), 6-*O*-[(6″-*O*-allophanyl)-β-D-glucopyranosyl]-2,5-dihydroxy-7-phenylphenalen-1-one (**10**), 6-*O*-[(6″-*O*-allophanyl)-β-D-glucopyranosyl]-5-hydroxy-2-methoxy-7-phenylphenalen-1-one (**11**).

50% of the compounds isolated from the leaves and the stem, but was only a minor component in the roots. Moreover, there were compounds which were found exclusively either above or below the ground, for example, **1** and **8** in the shoot and **6**, **10**, and **11** in the roots. A similar but less dramatic difference was observed for compound **4**. The difference between the shoots and the roots was obvious not only in the proportions but even more with respect to the total concentration of all compounds (Fig. 2b). An increasing concentration gradient was observed from the leaves via the stem to the root system. Compared with the leaves, an up to 16 fold higher concentration was determined in the root tips. This was twice as much as found in the mature part of the roots. The highest molar concentration of an individual compound was calculated for the phenylbenzoisochromenone derivative **3** with 20 μmol g^{-1} fr. wt in the root tips.

2.2. Distribution pattern in leaves

More detailed studies were performed on the distribution of seven selected compounds (**1**–**3**, **5**, and **7**–**9**) in the leaves. A comparison of the leaves of different age indicated that the patterns of phenylphenalenone-type compounds in the younger leaves (up to the 6th youngest leaf) differed only slightly (Fig. 3A–C), whereas a noticeable change in the concentration and relative abundance of compounds was observed in the older ones (Fig. 3D). The concentration of glucosides **7**–**9**, especially compound **7**, dropped dramatically with the age of the leaves while the aglycons simultaneously accumulated to somewhat higher levels.

Analysis of the concentrations and relative abundance of these seven phenylphenalenone-related compounds **1**–**3**, **5**, and **7**–**9** within individual leaves revealed significant differences. Decreasing amounts of glucosides **7**–**9** from the leaf base to the top were observed (Fig. 4). In contrast, the highest concentration of the aglycon **3** was found in the upper part of the leaves. In some samples, lowest levels, especially of **1**, were detected in the middle part of the leaves. However, the total molar concentration of all compounds (0.56 μmol g^{-1} fr. wt) was highest in the samples close to the leaf base, which was mainly due to the high levels of glycosides **7**–**9**.

2.3. Distribution in reproductive organs

The distribution pattern of phenylphenalenone-type compounds was clearly different in various parts of the inflorescence (Fig. 5). An exception was glucoside **7**, which, in general, was the most abundant compound (18 μmol g^{-1} fr. wt in the stamen). Similarly, allophanyl glucosides **8** and **9** occurred in all parts of the inflorescence. The aglycon **3**, an abundant compound in all other plant parts including peduncles and secondary axis of the inflorescence, was absent from the typical reproductive organs like carpels, stamen, and petals. The oxabenzochrysenone derivative **5** was found to be one of the major components in carpels, as a minor component in the stamen, but was lacking in petals,

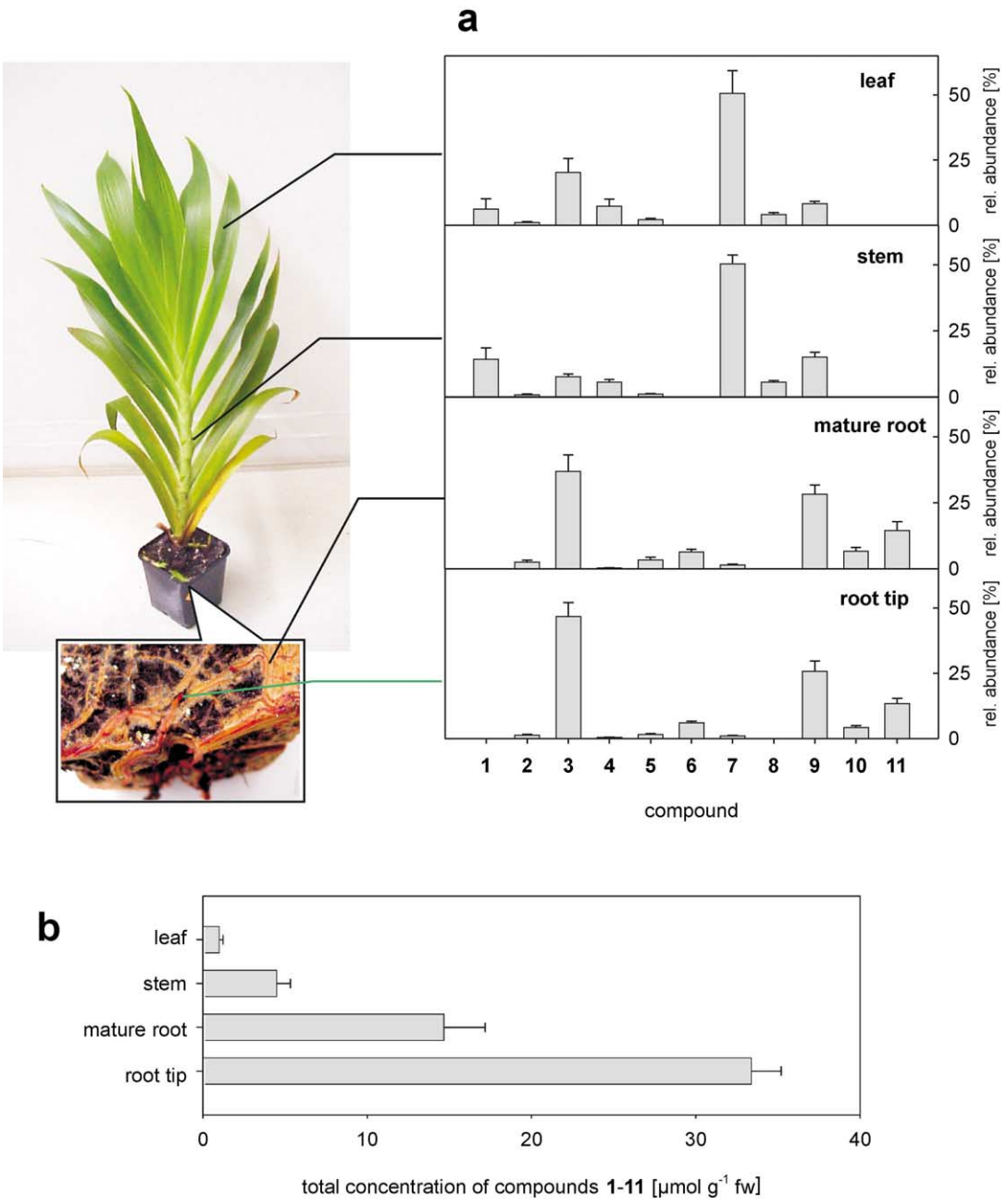

Fig. 2. Distribution of phenylphenalenone-type compounds **1**–**11** in vegetative organs of *Xiphidium caeruleum*. (a) Mean relative abundance (±S.E., $N=6$; total concentration = 100%) and (b) mean total concentration (±S.E., $N=6$) within plant organs.

secondary axis of inflorescence, and peduncles. Compounds **2**, **6**, **10**, and **11** were below the detection level in the inflorescence. By contrast to the flowers, the peduncle and secondary axis of inflorescence exhibited an overall pattern similar to that of the green vegetative organs, and the total concentration closely resembled that of the stem (Fig. 2). The highest total molar concentration (30 µmol g^{-1} fr. wt) was calculated for the stamen (Fig. 5b), which was comparable with that in the root tips (Fig. 2). HPLC analysis indicated the occurrence of relatively high amounts of hitherto unidentified phenylphenalenone-type compounds in all parts of the flowers.

3. Discussion

This work was aimed at elucidating the distribution of phenylphenalenones and related compounds in *Xiphidium caeruleum*, a species of the Haemodoraceae. Phytochemical studies (Cremona and Edwards, 1974; Opitz et al., 2002) had revealed the occurrence of compounds

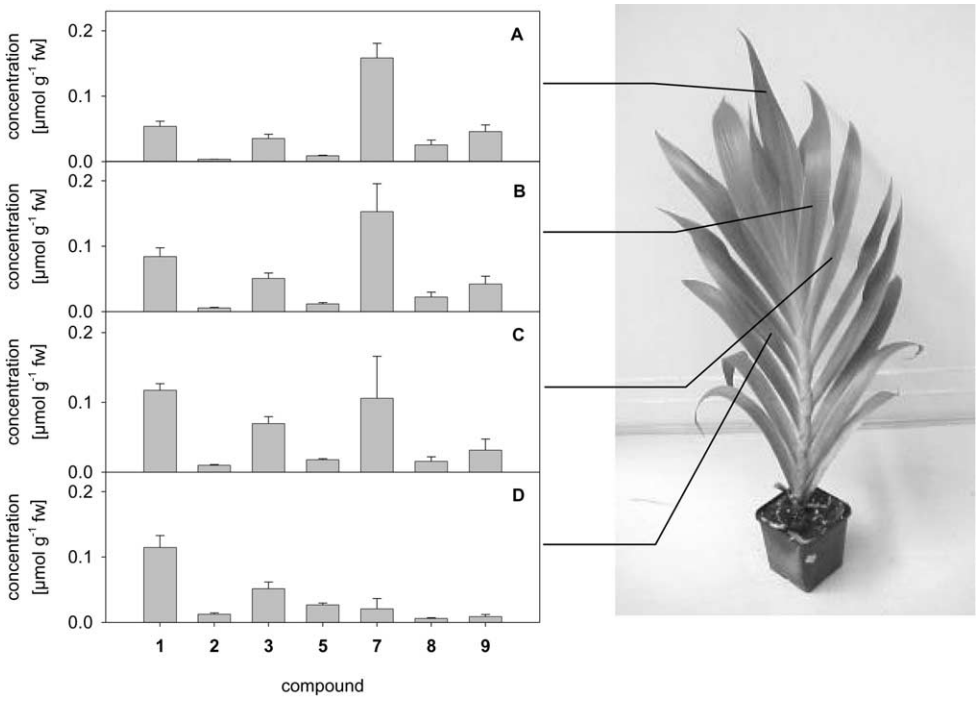

Fig. 3. Mean molar concentrations of phenylphenalenone-type compounds **1**–**3**, **5**, and **7**–**9** in leaves of different age of *Xiphidium caeruleum* plants. (A) Second, (B) fourth, (C) sixth, and (D) ninth youngest leaf.

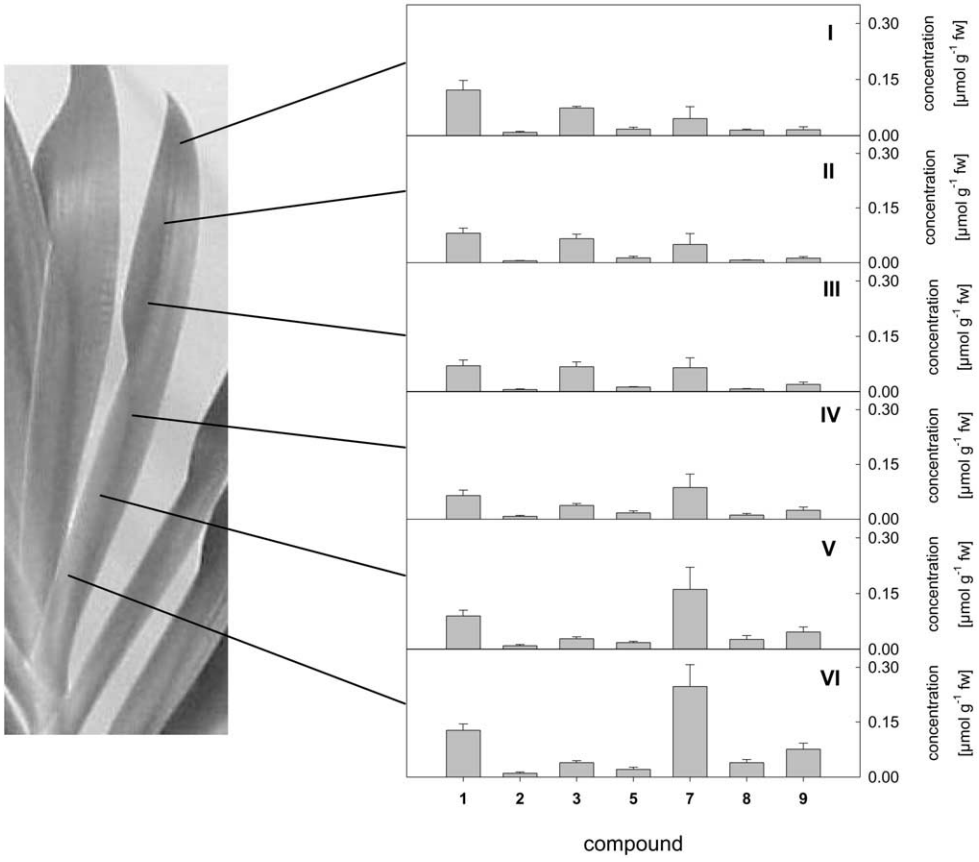

Fig. 4. Mean molar concentrations of phenylphenalenone-type compounds **1**–**3**, **5**, and **7**–**9** of *Xiphidium caeruleum* leaves. Samples I–VI were taken from leaf sites 6 cm distant from each other.

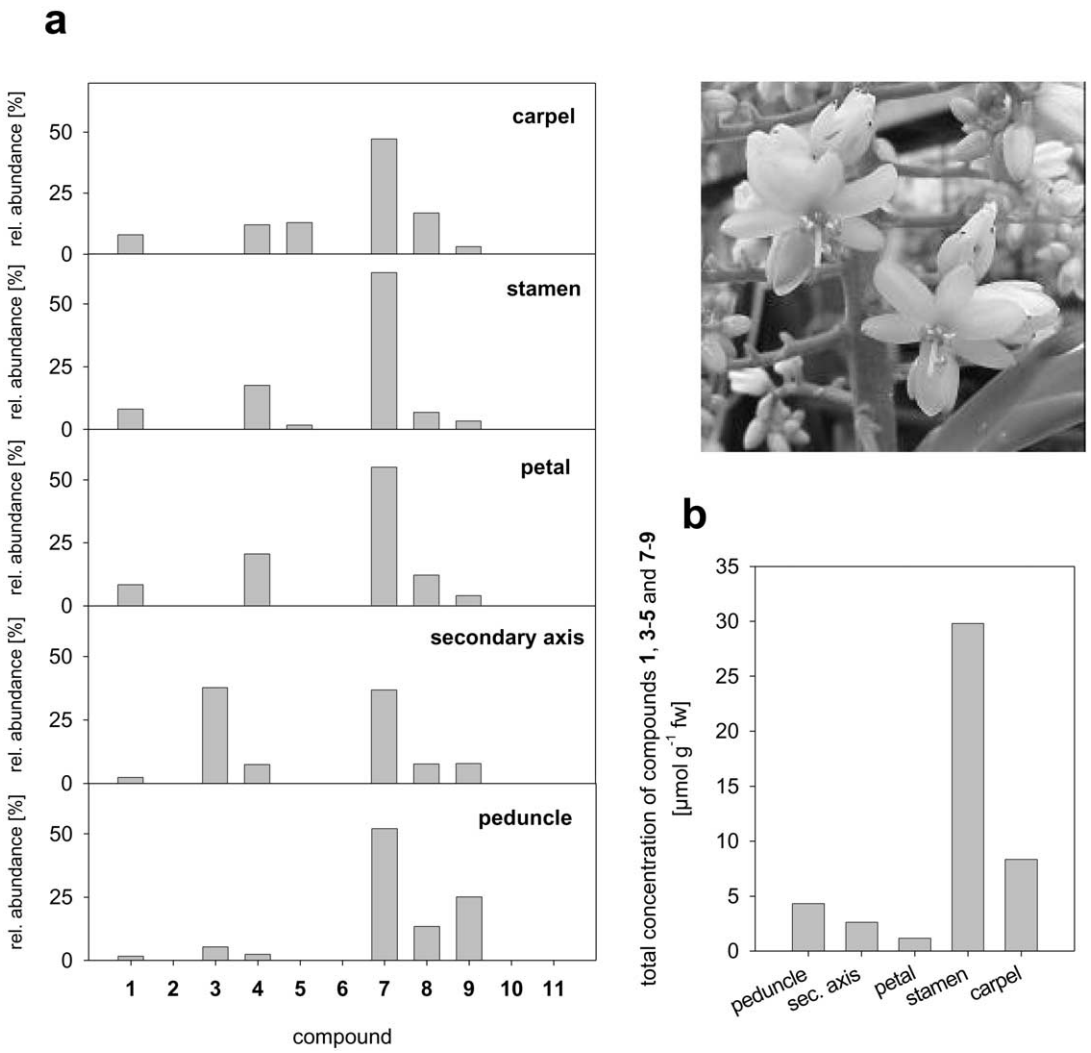

Fig. 5. Distribution of phenylphenalenone-type compounds **1**–**11** in reproductive organs of *Xiphidium caeruleum*. (a) Relative abundance (total concentration = 100%) and (b) total concentration of compounds **1**–**3**, **5**, and **7**–**9**.

of that type throughout the whole plant. The results presented here indicate highest total concentration in the root system with an increasing gradient toward the youngest parts (root tips) and, within the reproductive organs, in the stamen. Accumulation of phenylphenalenone-type compounds in young plant parts and generative organs is in accord with frequently reported data on other secondary metabolites such as terpenoids (Gershenzon and Croteau, 1991), different types of alkaloids (Hartmann and Zimmer, 1986; Laus et al., 1997), glucosinolates (Porter et al., 1991), and flavonoids (Fico et al., 2000). Although it is difficult to generalize from the data available, levels of secondary metabolites are often higher in young tissue and/or reproductive organs, suggesting a role of these compounds in plant defence. From these data, a protective function of phenylphenalenone-type compounds with beneficial impact for the plant seems to be likely.

Analysis of vegetative and reproductive organs with respect to the abundance of individual components, and comparison of different leaves and different sites within single leaves revealed accumulation of specific compounds in these plant parts: 6-hydroxy-5-methoxy-7-phenylbenzoisochromenone (compound **3**) and its allophanyl glucoside **9** were the most abundant compounds in the roots and also occurred, although in much lower concentration, in the other plant organs. Conversely, glucoside **7**, a minor compound of the roots, was abundant in the leaves (except old ones), stems, and all parts of the inflorescence. The specific function of these compounds in different tissues requires further studies.

The aglycon moieties of allophanyl glucosides **10** and **11** represent typical phenylphenalenones containing an intact C-19 carbon skeleton. Although converted to an oxabenzochrysenone system, the same is true for compound **6**. These three compounds occurred exclusively in the roots and were not found in detectable concentration in the aerial plant parts. By contrast, all metabolites detected in the leaves, stem and inflorescense belong to the phenylbenzoisochromenone

subtype, which has been suggested to be formed by oxidative rearrangement from precursors containing the C-19 skeleton of phenylphenalenones (Edwards and Weiss, 1974; Opitz et al., 2002). This putative biosynthetic relationship, together with the partially separated occurrence of the phenylphenalenone and phenylbenzoisochromenone subgroups, suggests biosynthesis in the roots and translocation toward the aerial plant organs. However, this hypothesis has to be confirmed by further investigations.

The absence of xiphidone, which previously has been reported as the major phenylphenalenone from *X. caeruleum*, in any of the plant organs studied here, may indicate some variability of the natural product pattern in this species. Different growing conditions, seasonal or other periodical changes could be responsible for this variation.

4. Experimental

4.1. Plant material and sampling

Plants of *Xiphidium caeruleum* (Aublet) were obtained from the University of Bochum (Botanical Institute) and maintained at the Botanical Garden of the University of Jena. Plants were transferred to growth chambers for variable time intervals at 28/15 °C day/night temperature, 50/85% relative humidity and a daily 14-h light period. Plants grown in the growth chamber were used in all experiments except those on reproductive organs. For distribution studies on vegetative organs, three similar sized and aged plants of *X. caeruleum* were studied. Using a razor blade, two cross section samples were taken from two different leaves and two from the stems of each plant. The root tips and the segments of the mature root sections (approximately 5 cm distant from the tip) of the same three plants were taken as well (Fig. 2). The distribution in leaves of four different ages (2nd-, 4th-, 6th- and 9th youngest leaf, Fig. 3) and at different positions of the leaf (Fig. 4) was studied. In the latter experiment, six cross sections (three samples from the lamina and three samples from the blade) were taken from sites 6 cm distant from each other. The top sample from the lamina was taken approximately 6 cm behind the leaf tip. To study distribution in flowers, inflorescences (Fig. 5) were harvested from greenhouse grown plants. Appropriate parts of the flowers were pooled for analysis resulting in samples of six carpels, 20 stamen, and six petals. In addition samples were taken from the peduncles.

4.2. Extraction, HPLC analysis and quantification

Each plant sample was placed into a 2.0 ml screwtop microcentrifuge tube containing Lysing Matrix D (0.9 g; Q-Biogene, Heidelberg, Germany). To each tube, MeOH (1 ml) and pyrene (10 μg) (Aldrich) as an internal standard were added. The mixture was homogenized for 45 s at 6.5 m s^{-1} using the FastPrep® System (FP120; Q-Biogene, Heidelberg, Germany). The homogenate was centrifuged and the supernatant was transferred into a new vial. The cell debris was extracted once again with MeOH (1 ml). Both methanolic extracts were pooled, dried (Concentrator 5301, Eppendorf, Germany), and frozen at −80 °C. Aliquots of the dried samples were dissolved in DMSO (100 μl) for reversed-phase HPLC analysis on a LiChrospher 100 RP-18 column (5 μm; 250×4 mm) using a linear MeCN-H_2O gradient (0.1% TFA) 5%→65% MeCN in 50 min with a flow rate of 0.8 ml min^{-1} and DAD detection (monitoring wavelength 254 nm). Compounds **1**–**11** were identified by means of comparison of retention times (**1**, R_t 43.0 min; **2**, R_t 42.6 min; **3**, R_t 44.8 min; **4**, R_t 33.3 min; **5**, R_t 38.0 min; **6**, R_t 40.4 min; **7**, R_t 24.9 min; **8**, R_t 26.3 min; **9**, R_t 26.6 min; **10**, R_t 26.2 min; **11**, R_t 25.8 min) and UV-VIS spectra with those of authentical reference compounds (Opitz et al., 2002). Lachnanthopyrone (**1**) and compound **5** have been described first from *Lachnanthes tinctoria* (Edwards and Weiss, 1972, 1974). The concentration was calculated on the basis of the integral of the UV absorption curve obtained from the HPLC. Calibration curves of compounds proved the linearity of the concentration-absorption ratio up to a concentration of 0.02 μM in the HPLC sample. Within the experiments described here, concentrations of individual compounds did not exceed this level.

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