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Direct emission of the allergen primin from intact *Primula obconica* plants

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The allergen primin and its precursor miconidin were collected by dynamic headspace technique from *Primula obconica* Hance and quantified and identified by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The primin concentrations in leaves/stems and flowers (including pedicel and calyx) of *P. obconica* were 262 μg and 531 μg (g fresh plant material)$^{-1}$, respectively, whereas miconidin concentrations were 92 μg (g fresh plant material)$^{-1}$ in leaves/stems and 194 μg (g fresh plant material)$^{-1}$ in flowers. The amounts of primin released from unchopped leaves/stems and flowers were 65.3 ng and 18.8 ng (g fresh plant material)$^{-1}$ h^{-1}, respectively. Dynamic headspace analysis of intact *P. obconica* plants showed that primin is also directly emitted from undamaged plants, although in smaller concentrations (6.2 ng (g fresh plant material)$^{-1}$ h^{-1}), whereas miconidin was only emitted in minute amounts (less than 0.4 ng (g fresh plant material)$^{-1}$ h^{-1}) both from intact plants and from unchopped plant parts. The possibility that primin from *P. obconica* could be a potential airborne allergen and therefore a source of airborne contact dermatitis is discussed.

Key words: Primula obconica; Primulaceae; primin; miconidin; allergen; dynamic headspace analysis; plants; allergic contact dermatitis; airborne; occupational; horticulture; floristry. © Munksgaard, 2000.

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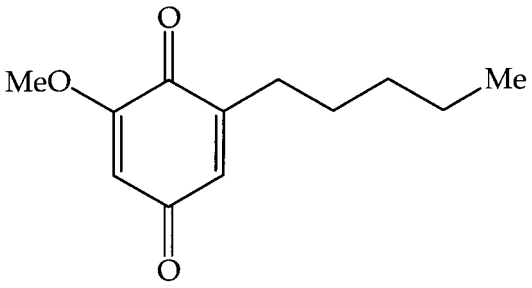
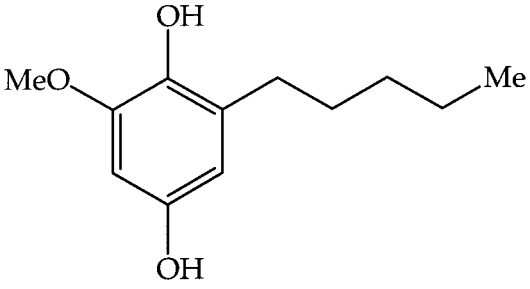
Primula obconica Hance is a very popular plant for heated glasshouses and is by far the most successful primula as a house plant, primarily because it is remarkably tolerant of modern central heating (1). Unfortunately, *P. obconica* is the most allergenic among primulas and has long been known as a significant source of allergic contact dermatitis in Europe, especially in England, Germany, and the Scandinavian countries, and consequently there have been and still is some reluctance by plant breeders and nurseries, in, e.g., Denmark, to grow *P. obconica*. However, according to data from the Aalsmeer flower market in Holland, the production of *P. obconica* and other primulas has not declined (2).

The sensitizing properties of *P. obconica* are primarily due to the accessibility of the allergen primin (2-methoxy-6-pentyl-1,4-benzoquinone, Fig. 1) on the surface of the plant (3–7). Primin is formed in minute glandular hairs (trichomes), consisting of 3–5 cells, and is accumulated between the cuticula and the cell membrane of the outermost cells (3–7). When the cuticula burst, the concentrated allergen accumulates as irregular resinous drops on the top and sides of the hair. The highest concentrations of primin are normally found on the calyx and decrease in the following order: pedicel (flower stalk), carpel, leaves, petiole, and inflorescent stem (3–6). However, recent investigations of *P. obconica* have also revealed the presence of miconidin (2-methoxy-6-pentyl-1,4-dihydroxybenzene, Fig. 1), the biosynthetic precursor of primin, in the trichomes, together with other benzoquinones and dihydroxybenzenes (8–10). Miconidin is also a major constituent in the trichomes and is most likely allergenic (8). Therefore, contact with *P. obconica* always bears risk of sensitization, e.g., the removal of dead flowers from the plant involves direct contact with the the calyx and the pedicel. This is probably how most people (e.g., housewives, nurserymen, and florists) become sensitized (2, 3, 6, 11). Indirect contact with *P. obconica* via, e.g., door handles or handshakes can also elicit an allergic reaction (3, 6). Furthermore, small plant parts or dust particles containing primin can be released from *P. obconica* plants, resulting in air-



Primin



Miconidin

Fig. 1. Chemical structures of primin and miconidin.

borne contact dermatitis (6, 12). Finally, it has been suggested that primin may be directly emitted from *P. obconica* (3, 6) and consequently could elicit an allergic reaction in highly-sensitized persons.

The possibility that primin is directly released from intact *P. obconica* plants and/or unchopped plant parts was investigated in order to evaluate the potential of primin as a possible airborne contact allergen.

Materials and Methods

Plant material

Plants of *Primula obconica* Hance were obtained in July from the nursery Hyldehøj (Kauslunde, Denmark) in the flowering stage and ready for sale.

Collection of volatiles from P. obconica by dynamic headspace analysis

For collection of primin and other volatiles by dynamic headspace analysis from intact *Primula obconica* plants; 1 pot plant (flowering stage; fresh weight 80–95 g) was carefully guided into a 25-litre glass bulb with a 2-flange lid without stoppers. For collection of volatiles from unchopped leaves/stems and flowers (including pedicel and calyx), respectively, a 4-litre glass bulb was used. Collection was made from 25–35 g fresh unchopped leaves/stems or flowers in each investigation. Subsequently, the glass bulbs were closed airtight with Teflon stoppers mounted with Teflon tubing (3.2 mm o.d.). The air flow in the glass bulbs was 300 ml min^{-1} and was achieved by a Miniport N 75 KN18 vacuum pump (KNF Neuberger, Freiburg, Germany). The incoming air was purified by passing it through a cylindrical steel container containing 250 ml activated charcoal granules (ML gas puritube, Mikrolab, Aarhus, Denmark). The experiments were conducted in the laboratory without artificial light and the air temperature in the glass bulbs was ca 20°C (room temperature). The volatiles were collected in glass tubes (18 cm×4 mm i.d.) equipped with 200 mg Porapak Q 50–80 mesh (Waters Inc., Milford, Massachusetts, USA) inserted between 2 desiloxanized glass wool plugs. Collection time was 8 h unless otherwise specified. Volatiles were eluted from the Porapak columns, with 3-ml double-distilled dichloromethane (CH_2Cl_2), into 4-ml glass vials. For quantification, eugenol (5500 ng in 5 μl CH_2Cl_2) was transferred with a Hamilton syringe to the vials prior to careful evaporation of excess solvent under nitrogen flow to a final volume of 200 μl.

The equipment (glass bulbs, lid, tubings, stoppers and Teflon connectors) was cleaned and tested for impurities between each sampling, as described previously (13).

Extraction of primin and miconidin from P. obconica

Fresh unchopped leaves/stems (20–30 g) and flowers (20–30 g) of *P. obconica* were extracted 2× with CH_2Cl_2 (150 ml) for 25 min, and the primin and the miconidin content in the combined extracts were determined by GC and GC-MS as described below.

Analysis for primin and miconidin in headspace samples and extracts by GC and GC-MS

Gas chromatography was used for quantification of primin and miconidin in headspace samples and extracts, and was performed on a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a Restek crossbond (Hewlett-Packard) capillary column (30 m×0.25 mm i.d., d_f=0.25 μm liquid phase: Rtx®–20). Helium was applied as carrier gas with a flow rate of 1 ml min^{-1} and 70 kPa (10 psi) column head pressure. The oven temperature was programmed to be isothermal for 2.5 min at 80°C and to increase from

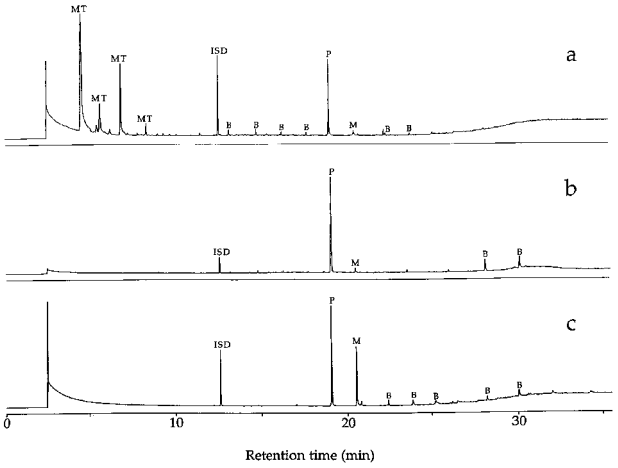


Fig. 2. GC-MS chromatograms of headspace samples of *Primula obconica* (a) intact plant, (b) leaves/stems and (c) a dichloromethane extract of fresh unchopped leaves/stems of *P. obconica*. ISD=internal standard, eugenol; MT=monoterpenes; P=primin; M=miconidin; B=background contaminants. For chromatographic conditions see *Materials and Methods*.

80°C to 300°C at a rate of 8°C min^{-1}, and subsequently isothermal at 300°C for 15 min. The injection temperature was 200°C and the FID detector temperature 230°C. 2 μl of each sample were injected onto the GC column in splitless mode (splitless purge time=60 s). The primin and the miconidin contents in the Porapak eluates and extracts were estimated from FID peak areas of primin and miconidin and the standard, eugenol (Aldrich, Steinheim, Germany), used in the GC analysis. The response factor was set to 1 for primin, miconidin, and the internal standard. Other volatile compounds (mainly monoterpenes) were not quantified.

A Varian Saturn 2000 ion trap mass spectrometer operated at 70 eV and interfaced to a Varian Star 3400 CX gas chromatograph equipped with a Rtx®–20 capillary column was used for GC-MS. The GC conditions were the same as described above. Identification of primin and miconidin was made from GC retention data (Fig. 2) and by comparing the mass spectrum of the entrained primin and miconidin with authentic samples (Table 1) isolated from *P. obconica* by column chromatography and identified by NMR (^1H and ^{13}C), as described previously (8). Other compounds in the headspace were identified as common monoterpenes (e.g., α- and β-pinene, 3-carene) and were released only from the flowers (Fig. 2).

Results

The amounts of primin and miconidin in leaves/stems were 262 μg and 92 μg (g fresh plant material)$^{-1}$, respectively, and in flowers the concentrations were 531 μg and 194 μg (g fresh plant material)$^{-1}$, respectively. The amount of primin directly emitted from intact *P. obconica* was 6.2 ng (g fresh plant material)$^{-1}$ h^{-1}, whereas the amounts emitted from unchopped leaves/stems and flowers were 65.3 ng (g fresh plant material)$^{-1}$ h^{-1} and 18.8 ng (g fresh plant material)$^{-1}$ h^{-1}, respectively. Only minute amounts of miconidin (less than 0.4 ng (g fresh plant material)$^{-1}$ h^{-1}) were emitted from *P. obconica* (Table 1).

Discussion

Airborne contact dermatitis (ABCD) is caused by airborne allergens and/or irritants, and usually appears on areas exposed to air. Not only the face but also areas of the skin where allergens/irritants can be trapped are critical, for example, the eyelids, neck, forearms, and/or lower legs (14, 15). Plants are a rich source of airborne allergens and irritants and consequently airborne contact dermatitis caused by plants is common. Airborne plant allergens and/or irritants are often spread into the air via pollen or dust particles (14, 15), although volatiles with low allergenic potential, such as eugenol, isoeugenol, limonene, and α-pinene (16), are known to be emitted from many plant species (17) and to be well-known constitu-

Table 1. The content of primin and miconidin in *Primula obconica* and the amounts emitted from leaves/stems, flowers, and intact plants

	Emitted amounts (ng (g fresh plant material)$^{-1}$ h^{-1})[a]			Content (μg (g fresh plant material)$^{-1}$)[a]		
Compounds	leaves/stems	flowers	intact plants	leaves/stems	flowers	mass spectral ions *(m/z)*[b]
primin	65.3±32.8	18.8±10.3	6.2±0.6[c]	262±37	531±163	153, 124, **208**, 109, 69, 179, 39, 53, 41, 95, 139, 165, 81, 193
miconidin	0.4±0.3	0.1±0.1	0.1±0.1	92±9	194±88	154, **210**, 139, 125, 122, 110, 69, 167, 39, 53, 94, 41, 181, 195

[a] Values given as mean±standard deviation (n=3).
[b] Fragmentation ions, base peak, and characteristic ions in decreasing order of relative abundance. Molecular ion boldface.
[c] Emission corresponds to approximately 580 ng $plant^{-1}$ h^{-1}.

ents in natural and synthetic fragrances (16). However, the direct release of potent contact allergens from plants has so far only been described for tulipalin A from *Alstroemeria* cut flowers (13). In the latter case, tulipalin A may be responsible for some cases of ABCD caused by *Alstroemeria* (13).

Primula dermatitis is mainly caused by *P. obconica* and has variable clinical manifestations that are often misdiagnosed, since patients usually do not present with a typical picture of plant dermatitis with blotches and linear streaks on the skin (11, 18–24). Consequently, the allergen primin has been included in the European standard series (20). The degree of sensitivity and the intensity of exposure determine the pattern of dermatitis. The main pattern of primula dermatitis is facial dermatitis, alone or in combination with limb or hand dermatitis (3, 6, 11, 12, 18–24). Most cases of primula dermatitis are due to direct contact with the plants, whereas only a few cases of airborne contact dermatitis have been described (3, 6, 12, 15). However, some highly-sensitized patients may develop an eruption if they are in a room with *P. obconica*, in spite of careful avoidance of direct contact with the plant (3, 6). This could be explained by indirect contact via, e.g., a doorhandle, or because the allergen primin is directly released from the plant. The latter hypothesis has so far not been proven nor even investigated.

The primin concentrations in leaves/stems and flowers of *P. obconica* were 262 μg and 531 μg (g fresh plant material)$^{-1}$, respectively, whereas miconidin concentrations were 92 μg (g fresh plant material)$^{-1}$ in leaves/stems and 194 μg (g fresh plant material)$^{-1}$ in flowers. Headspace experiments with pure primin (collection time 5 h) showed that this compound is entrained by headspace technique, indicating that primin may be directly emitted from *P. obconica* into the air. The same experiments with miconidin revealed only trace amounts of miconidin in the headspace samples and this compound is therefore not likely to be emitted from the plants in significant amounts. Furthermore, no primin was detected in the headspace experiments with miconidin, indicating that miconidin is not oxidized to primin in significant amounts under our experimental conditions. Dynamic headspace analysis performed on intact *P. obconica* and subsequent analysis of the samples by GC and GC-MS confirmed that primin is directly emitted from *P. obconica* in significant amounts, in contrast to miconidin (Table 1, Fig. 2). The amount of primin emitted from an intact plant was 6.2 ng (g fresh plant material)$^{-1}$ h^{-1}, corresponding to approximately 580 ng plant^{-1} h^{-1}, whereas the amounts emitted from unchopped leaves/stems and flowers were 65.3 ng and 18.8 ng (g fresh plant material)$^{-1}$ h^{-1}, respectively. The higher amounts emitted from the unchopped plant parts are probably due to the release of primin from damaged trichomes prior to headspace sampling, compared to intact plants. The lack of correlation between the emission of primin from unchopped leaves/stems and flowers, and the content in the respective plant parts (Table 1), probably reflects the fact that the trichomes more easily burst in leaves/stems, compared to flowers, prior to and during headspace sampling. The emission from intact plants can be compared with the direct emission of primin from undamaged *P. obconica* and shows that the trichomes also burst under these circumstances.

The amount of allergen formed in *P. obconica* varies considerably, depending on season, photoperiod, cultivation practice, and the horticultural variety (3, 6, 7, 18). The primin content is, for example, highest during a warm summer and lowest during winter, when a plant may give negative patch test reactions in a primin-sensitive person. The primin content in the investigated *P. obconica* is comparable with the amounts found in other investigations of *P. obconica* in the spring/summer (7, 8), and consequently the emitted primin concentrations may reflect the amounts generally released from *P. obconica* at this time of the year.

Although the amounts of primin emitted from *P. obconica* are low compared to the content present on the surface of the plant, the emitted primin concentrations are comparable with the tulipalin A concentrations emitted from *Alstroemeria* cut flowers (13). Airborne tulipalin A may elicit an allergic reaction in highly-sensitized persons and may consequently play an important rôle in relation to ABCD caused by these plants (13). It is therefore not unreasonable to assume that airborne primin could play a similar rôle with regard to primula dermatitis. Based on the present investigation, we suggest that airborne primin should be taken into consideration when investigating the possibilities of primula dermatitis, in particular ABCD.

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