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Secondary Metabolites from the Phloem of *Piper solmsianum* (Piperaceae) in the Honeydew of *Edessa meditabunda*

Clécio S. Ramos[a]* and Massuo J. Kato[b]

ABSTRACT:
Introduction – The phytochemistry of species of the genus *Piper* has been studied extensively, including *Piper solmsianum*. However, no studies have addressed the phytochemistry of the sap content of *Piper* species.
Objective – To evaluate the transferring of secondary compounds from the saps of *P. solmsianum* to the honeydew of *Edessa meditabunda*.
Methodology – The honeydew of *E. meditabunda* and saps of *P. solmsianum* were analysed by GC-MS, ^{1}H-NMR and LC-MS.
Results – The lignan (−)-grandisin and the phenylpropanoid (*E*)-isoelemicin were detected in both saps of *P. solmsianum* and honeydew of *E. meditabunda*.
Conclusion – Analysis of honeydew secreted by the sap-sucking insect *E. meditabunda* indicated that (−)-grandisin and (*E*)-isoelemicin are absorbed from the phloem of *Piper solmsianum*.

Supporting information can be found in the online version of this article.

Keywords: Lignans; phloem; sap; Edessa meditabunda; Piper solmsianum; Piperaceae

Introduction

The Piperaceae family belongs to the super order Nymphaeiflorae, order Piperales, and comprises five genera and about 1400 species (Santos *et al*., 2001). Their species are of pantropical occurrence with species distributed in the Western Hemisphere from Mexico to southeastern Argentina. Brazilian forests harbour 238 species of *Piper* L., 25 species of *Ottonia* Sprengel, and two species of *Pothomophe* Miquel (Guimarães and Carvalho-Silva, 2009).

The phytochemistry of the Piperaceae species, mainly of the *Piper* genus (Parmar *et al*., 1997), has been studied extensively due to the variety of biological activities displayed by their secondary metabolites (Alécio *et al*., 1998; Navickiene *et al*., 2000; Martins *et al*., 2003; Lago *et al*., 2004). Phytochemical studies of Brazilian species of Piperaceae have revealed the presence of amides (Silva *et al*., 2002; Navickiene *et al*., 2003), neolignans (Benevides *et al*., 1999), lignans (Martins *et al*., 2000, 2003; Ramos *et al*., 2008) and prenylated benzoic acid and chromene (Baldoqui *et al*., 1999; Ramos and Kato, 2009; Ramos *et al*., 2009).

The leaves, roots, stems and inflorescences of *P. solmsianum* have been investigated previously and are known to contain one dihydrobenzofuran neolignan, one glycosylated flavonoid (Moreira *et al*., 1995), five phenylpropanoids and four tetrahydrofuran lignans (Martins *et al*., 2000, 2003), including (*E*)-isoelemicin and (−)-grandisin (Ramos *et al*., 2008). In spite of the studies carried out on different organs, no phytochemical investigation has been carried out on the sap content for any of the *Piper* species. Such analysis may provide evidence for translocation of secondary compounds along the plant and through *Piper*–insect interactions, such as by the sap-sucking insect *Edessa meditabunda*. The data may contribute to determining the flow of secondary compounds from the phloem to the honeydew secreted by the insects.

Experimental

General

The ^{1}H-NMR analysis was conducted at 200 MHz (Bruker AC-200) using deuterated chloroform ($CDCl_3$; Aldrich) and tetramethylsilane (TMS; Aldrich) as a solvent reference. Electron impact (EI) MS was measured at 70 eV in a HP 5990/5988A spectrometer. The electrospray ion source (ESI) was measured at 4 kV, on an Agilent Technologies model SL. The HPLC analyses of the extracts obtained were performed in an HP-1050 instrument using a reversed phase column (Supelco, C_{18}; 5 μm i.d., 4 × 250 mm) eluted in a gradient mode starting with acetonitrile:water 3:7 for 8 min; 3:7 to pure acetonitrile in 40 min, and detection at 254 nm. The GC-MS analyses were carried out in a Shimadzu GCMS-QP5050A with a DB-5 column (30 m × 0.25 mm, film thickness 0.25 mm) with the oven temperature programmed from 100 to 280 °C at a rate of 10 °C/min, and a carrier gas (helium) flow rate of 1 mL/min.

Plant material

The leaves of *P. solmsianum* were collected in the Campus of the University of São Paulo (Brazil) in September 2004. A voucher specimen (329676, *P. solmsianum*) is deposited in the Herbarium of the Jardim Botânico do Rio de Janeiro.

* Correspondence to: C. S. Ramos, Departamento de Ciências Moleculares, Universidade Federal Rural de Pernambuco, CP 52.171-030 Recife, PE, Brazil. E-mail: clecio@dcm.ufrpe.br

[a] Departamento de Ciências Moleculares, Universidade Federal Rural de Pernambuco, CP 52.171-030, Recife, PE, Brazil

[b] Instituto de Química, Universidade de São Paulo, CP 26077, 05513-970, São Paulo, Brazil

(−)-Grandisin and (*E*)-isoelemicin

The (−)-grandisin and (*E*)-isoelemicin were isolated from leaves of *P. solmsianum* according to previously reported procedures (Martins *et al*., 2000, 2003). Dried leaves (2 g) of *P. solmsianum* were milled and extracted with dichloromethane:methanol (2:1, 30 mL × 3), which after concentration in a vacuum yielded 0.12 g of crude extract. From this extract, 2 mg was dissolved in methanol and eluted in a C_{18} cartridge (Sep-Pack) prior to a HPLC analysis.

Stem sap collection

Sap was collected between 1400 and 1600 hours using a gentle vacuum to suck the vessel content from stem segments. Because a vacuum cannot pull air through the pit fields at the ends of the vessels, successive segments are cut from the stem to release the vessel contents.

Honeydew collection

Edessa meditabunda specimens collected from the stems of *P. solmsianum* were maintained in glass flasks, having two individuals each, for 2 days. The honeydew excreted by the insects was collected from the flask walls using methanol. The methanol solution was concentrated under vacuum yielding 1.3 mg of crude extract. The dried extract was dissolved in 1 mL of methanol for HPLC, LC-MS and GC-MS analysis.

Stem sap analysis

The sap extracted from the insects collected in the *P. solmsianum* stems was filtered (0.45 μm) and injected directly in the HPLC. For GC-MS and ^{1}H-NMR analysis, the sap (15 mL) obtained from the stems was extracted with ethyl acetate. The organic fraction was concentrated in a vacuum yielding 3.1 mg of crude extract. (−)-Grandisin and (*E*)-isoelemicin in the sap content were identified by comparison of their spectroscopic and chromatographic data (GC-MS, LC-MS, HPLC and ^{1}H-NMR) with authentic compounds previously isolated.

Insect material

The specimens of *E. meditabunda* were collected on the Campus of the University of São Paulo (Brazil) in September 2004 and identified by Dr Sérgio Antônio Vanin. The specimens were maintained in glass tubes to obtain the honeydew excreted. The dried insects (40 °C) were milled and the honeydew was extracted with dichloromethane:methanol (2:1, 10 mL × 3). The extract was concentrated under vacuum and diluted in methanol for chromatographic and spectrometric analysis. The GC-MS and HPLC analyses of the extracts were carried out using solutions at concentration of 2 mg/mL in methanol.

Results and discussion

The xylem and phloem saps were extracted from the stem of *P. solmsianum* (15 mL) and 10 μL were directly injected into a HPLC. The chromatographic profile of the sap showed two peaks (**1** and **2**) with similar time of retention to (*E*)-isoelemicin and (−)-grandisin, respectively, when compared with the chromatographic profile of the extract from *P. solmsianum* leaves (Fig. 1). The sap (15 mL) was fractioned with ethyl acetate and the organic fraction concentrated under vacuum and analysed via GC-MS (Fig. 2), LC-MS and by ^{1}H-NMR. The MS and ^{1}H-NMR data confirmed the identities of **1** and **2** as (*E*)-isoelemicin and (−)-grandisin, respectively (see Supporting Information). The ^{1}H-NMR spectrum of sap content revealed one singlet at δ 6.67 (H-2/H-2′, H-6/H-6′), two intense singlets at δ 3.87 (4 × OC$\underline{H}_3$/3,3′,5,5′) and at δ 3.84 (2 × OC$\underline{H}_3$/4,4′) corresponding to the methoxyl groups. The set of signals corresponding to the tetrahydrofuran moiety included a doublet at δ 1.09 (6H, J = 6.0 Hz, H-9/H-9′), a multiplet at δ 1.78 (2H, H-8/H-8′), a doublet at δ 4.66 (J = 7.9 HZ, H-7/H-7′). These data are consistent with those observed for (−)-grandisin. Additionally, minor signals at δ 6.58 (singlet), 6.11 (multiplet) indicated the presence of (*E*)-isoelemicin in the sap content of *P. solmsianum*. Moreover, the analysis by GC-MS revealed the presence of fatty acids such as oleic acid and palmitic acid in *P. solmsianum* saps.

The crude (xylem) and elaborated (phloem) saps from *P. solmsianum* were collected together and at first it was not possible to determine whether (*E*)-isoelemicin and (−)-grandisin occurred in the xylem or phloem. Nevertheless, the excretion of honeydew by sap-feeding insects corresponds to the ingestion of phloem sap (Mittler, 1958). Thus, specimens of the sap-sucking insect *E. meditabunda* were collected on leaves of *P. solmsianum* for chemical investigation. The analysis by HPLC and GC-MS of extracts from the body and honeydew excreted by these insect specimens indicated compounds **1** and **2** (Fig. 1) as constituents in the phloem sap from *P. solmsianum*.

The presence of (*E*)-isoelemicin and (−)-grandisin in the sap and leaves of *P. solmsianum* provides evidence for secondary metabolite transport in the phloem in *Piper* species. Although the transport of secondary metabolites in plants has been investigated previously (Molyneux *et al*., 1990), this is the first report of secondary metabolites in sap of Piperaceae species. Such a method represents an alternative procedure to study transport and storage of secondary metabolites, which is generally limited to cytochemical techniques, analysis of isolated cell compartments and tracer labelling procedures (Luckner *et al*., 1980).

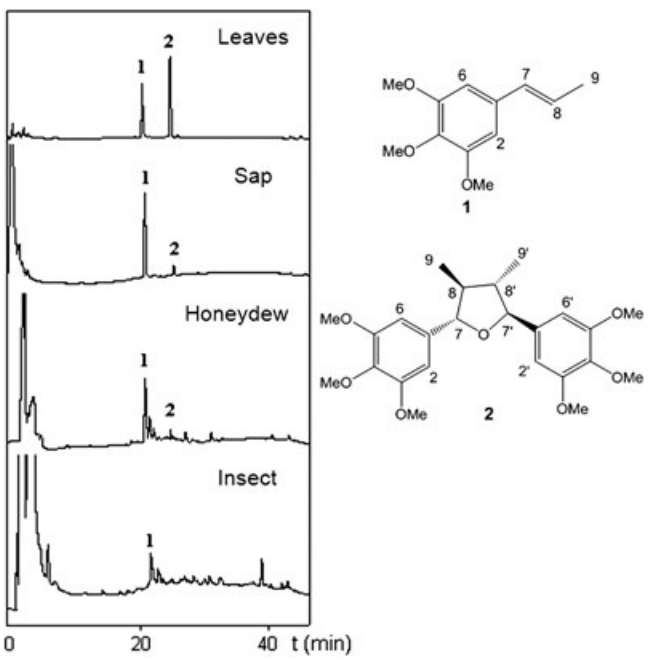

Figure 1. Chromatographic profiles (HPLC) recorded at 254 nm of the leaves and sap extracts from *P. solmsianum*, body and honeydew excreted of the *E. meditabunda* insect. Structures correspond to (*E*)-isoelemicin **(1)** and (−)-grandisin **(2)**.

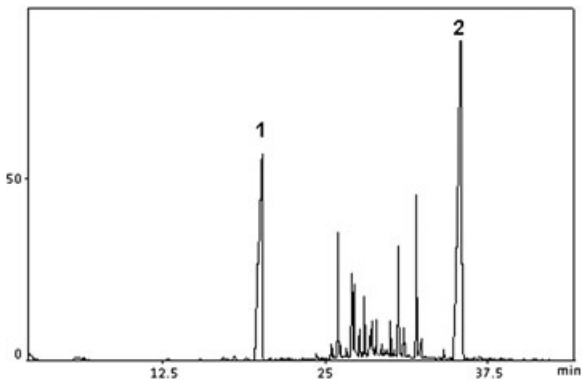

Figure 2. Chromatographic profiles (GC-MS) of sap content from *P. solmsianum*.

Supporting information

Supporting information can be found in the online version of this article.

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