Phytochemistry 69 (2008) 2157–2161

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Metabolism of (−)-grandisin from *Piper solmsianum* in Coleoptera and Lepidoptera species

Clécio S. Ramos [a], Sérgio A. Vanin [b], Massuo J. Kato [a,*]

[a] *Instituto de Química, Universidade de São Paulo, Av. Prof. Lineu Prestes, 748, 05508-900 São Paulo, SP, Brazil*
[b] *Museu de Zoologia e Instituto de Biociências, Universidade de São Paulo, Av. Nazaré, 481, 04263-000 São Paulo, SP, Brazil*

ARTICLE INFO

Article history:
Received 24 January 2008
Received in revised form 16 April 2008
Available online 17 June 2008

Keywords:
Piperaceae
Piper solmsianum
Lignans
Biotransformation
Naupactus bipes
Heraclides hectorides
Quadrus u-lucida

ABSTRACT

The biotransformation of the major *Piper solmsianum* leaf phenylpropanoids, such as the tetrahydrofuran lignan grandisin and derivatives was investigated in the beetle *Naupactus bipes* as well as in the caterpillars *Heraclides hectorides* and *Quadrus u-lucida*. Analysis of fecal material indicated that metabolism occurred mainly through mono- and di-*O*-demethylation at *para* positions of 3,4,5-trimethoxyphenyl rings of tetrahydrofuran lignans during digestion by these insects. Additionally, 3-hydroxy-4,5-dimethoxycinnamyl and 3,4,5-trimethoxycinnamyl alcohols were identified in fecal extracts of *N. bipes*.

1. Introduction

Lignans are widely spread in plant kingdom and have been described to present broad range of biological activities towards insects (Harmatha and Nawrot, 2002). The phenylpropanoid monomer safrol and the lignan sesamin, as well as other compounds bearing methylenedioxyphenyl groups, efficiently synergize the activity of pyrethroids, which lead eventually to development of piperonyl butoxide as a commercial synergist (Singh et al., 1976). Additionally, sesamin showed an antifeeding effect against caterpillars *Spilarctia oblique* (Srivastava et al., 2001), while lignans isolated from *Piper* species have shown antifeeding activity against stored grain pests (Nawrot and Harmatha, 1994). Naturally abundant and dietary lignans have been studied mainly in order to determine their roles and fates during metabolism and subsequent biological activities (Miyazawa, 2001; Smeds et al., 2007). The major biotransformation reaction of lignans in *Aspergillus niger* sp., rat intestinal bacteria and larvae of the insect *Spodoptera litura* involved aromatic ring *O*-demethylations, hydroxylations and glycosylations (Miyazawa et al., 1995, 2004; Miyazawa, 2001; Kasahara et al., 1996a,b).

Piper solmsianum, known popularly as "pariparoba", is a tropical shrub reaching up to 1–3 m height (Campos et al., 2005). Previous phytochemical studies carried out on leaves of one specimen of *P. solmsianum* yielded benzofuran neolignans and glycosylated flavonoids (Moreira et al., 1995), while detailed investigations carried out on roots, stems and inflorescences of different specimens indicated major accumulation of four tetrahydrofuran lignans and five phenylpropanoids, among which (−)-grandisin (**1**) and *E*-isoelemicin (**2**) were the major compounds, respectively (Martins et al., 2000, 2003).

During the past five years, several herbivorous insects have been observed predating on leaves of cultivated Piperaceae species including *P. solmsianum* (Vanin et al., 2008). Adult *Naupactus bipes* (Germar, 1824) beetles (Coleoptera, Curculionidae, Brachycerinae) appear to seek out and feed predominantly on leaves of *P. solmsianum* during the months from November to February. In the State of São Paulo (Brazil), eight species of *Naupactus* have been recorded as citrus trees pests; the adult insects feed on the leaves, whilst larvae avidly consume the roots. Species of *Naupactus* are considered to be largely polyphagous and no efficient pest control has been developed (Lanteri et al., 2002). Previous record on caterpillars assemblages predating on leaves of *Piper* species have been made on *P. aduncum* and *P. umbellatum*, two alien species, in Papua New Guinea rainforest (Novotny et al., 2003). In Southern Brazil, *P. amalago* is the main host of the *Heraclides hectorides* caterpillars (Pénz and Araújo, 1990). In our studies, caterpillars of three lepidopterous species, *H. brasiliensis* (Esper, 1794), *H. hectorides* (Rothsch and Jord, 1906) and *Quadrus u-lucida* (Plötz, 1884) on *P. solmsianum* and *P. regnellii*. These species are rich in tetrahydrofuran lignans have been observed (Martins et al., 2000, 2003) and

* Corresponding author. Tel.: +55 11 3091 3813.
E-mail address: majokato@iq.usp.br (M.J. Kato).

0031-9422/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2008.04.027

dihydrobenzofuran neolignans (Benevides et al., 1999), respectively (Vanin et al., 2008).

In the present study, the metabolism of *P. solmsianum* lignans by the beetle *N. bipes*, as well as *H. hectorides* and *Q. u-lucida* caterpillars have been investigated. The analysis of fecal materials by chromatographic and spectrometric methods revealed mono- and di-*O*-demethylation of the aromatic rings of grandisin (**1**) as the major biotransformation reactions yielding **1a** and **1b**, respectively. Additionally, 3,4,5-trimethoxycinnamic acid, 3,4,5-trimethoxycinnamyl alcohol and 3-hydroxy-4,5-dimethoxycinnamyl alcohol were isolated from *N. bipes* excretions.

2. Results and discussion

Heraclides hectorides and *Q. u-lucida* caterpillars and the beetle *N. bipes* feeding on *P. solmsianum* leaves appeared at our experimental site. In order to evaluate the metabolism of *P. solmsianum* leaves in *N. bipes* (adult insect), *H. hectorides* and *Q. u-lucida* (caterpillars), beetles and caterpillars were collected and maintained during one week on a diet of fresh *P. solmsianum* leaves in order to collect fecal material. Feces were dried and extracted with CH_2Cl_2:MeOH (2:1), and the same extraction was carried out with intact leaves. Fecal and leaf extracts were analyzed by HPLC and LC–MS, and the major compounds were isolated by chromatographic procedures and analyzed by NMR spectroscopy. Fecal extract chromatograms indicated the presence of additional peaks relative to those of leaf extracts (Fig. 1a–e). The major compounds observed in the leaves were the tetrahydrofuran lignan (−)-grandisin (**1**) and the phenylpropanoid (*E*)-isoelemicin (**2**) as previously described (Martins et al., 2000, 2003; Fig. 1a).

The chromatograms of *N. bipes* fecal extracts indicated a complete depletion of (*E*)-isoelemicin (**2**) but a remaining amount of (−)-grandisin (**1**) (Fig. 1b). Compounds corresponding to peaks **1a**, **1b**, **2a**, **2b** and **3a** were also identified but they were absent in the chromatogram of intact leaves (Fig. 1a). The compounds corresponding to the peaks **1a**, **1b**, **2a**, **2b** and **3a** were purified and their structures determined.

The EIMS spectra of compounds **1a** and **1b** presented molecular ions at *m*/*z* 418 and *m*/*z* 404 which are 14 and 28 units lower than the EIMS spectrum of (−)-grandisin (**1**, *m*/*z* 432), respectively. The fragmentation pattern of compounds **1a** and **1b** were in agreement with either regioisomers of *O*-demethylgrandisin or di-*O*-demethylgrandisin, respectively. The demethylation reactions affording compounds **1a** and **1b** were confirmed to take place at the *para*-methoxyl groups based on the aromatic ring symmetry observed in ^{1}H NMR spectrum and supported by literature data (Barbosa-Filho et al., 1989). Further confirmation for the identity of lignan **1b** was provided by its mass spectrum and retention time in HPLC and GC, relative to those of an authentic compound synthesized according to a procedure previously described (Sarkanen and Wallis, 1973). Thus, compounds **1a** and **1b** were identified as 4-*O*-demethylgrandisin and 4,4′-di-*O*-demethylgrandisin, respectively, and were determined to be biotransformation products of (−)-grandisin (**1**) by *N. bipes* (Fig. 2).

The EIMS data for compound **2a**, the major constituent in *N. bipes* feces, presented a molecular ion peak at *m*/*z* 224 compatible with a molecular formula of $C_{12}H_{16}O_4$. The compound was analyzed by IR, ^{1}H and ^{13}C NMR spectroscopies, and identified as 3,4,5-trimethoxycinnamyl alcohol (Sadik et al., 2003).

The ^{1}H NMR data obtained for compound **2b** resembles those for compound **2a**. Nevertheless, its EIMS data showed a molecular ion peak at *m*/*z* 210 compatible with a demethylated derivative of **2a**. Its ^{1}H NMR spectrum had signals corresponding to two aromatic hydrogens splitting to two doublets at δ 6.66 (1H, *J* = 2.2 Hz, H-6) and δ 6.51 (1H, *J* = 2.2 Hz, H-2), which were in agreement

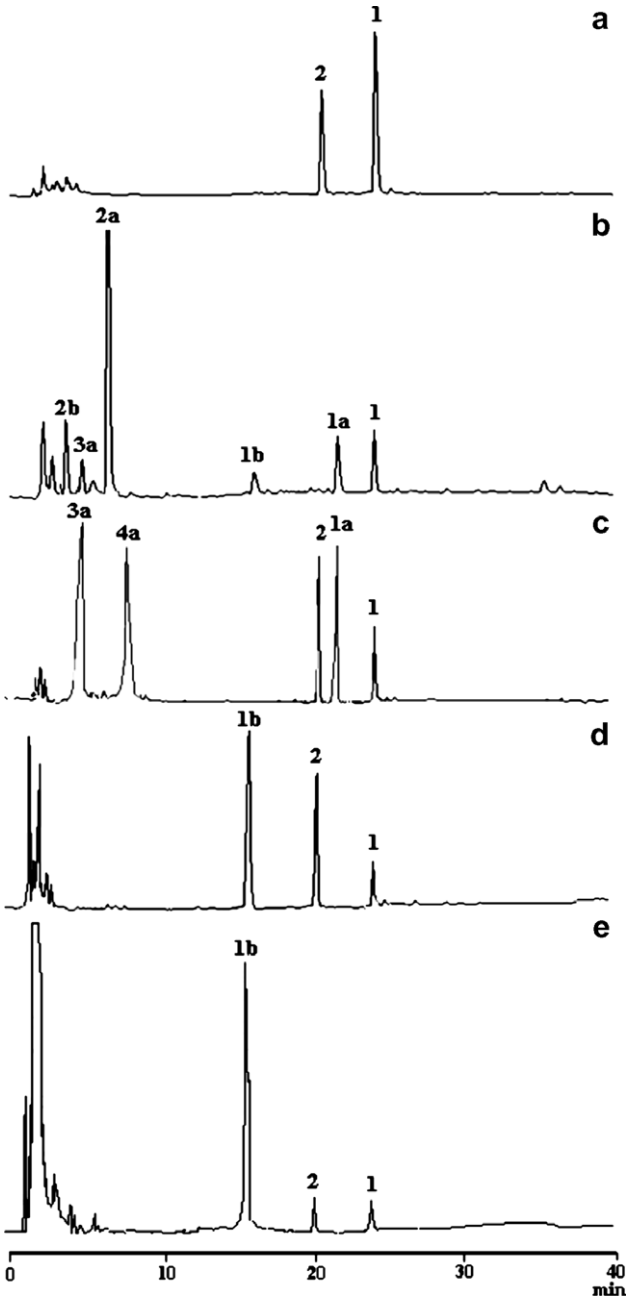

Fig. 1. Chromatographic profiles (HPLC) of *P. solmsianum* leaf extracts and insect fecal extracts. (a) *P. solmsianum* leaves; (b) *N. bipes* feces; (c) *H. hectorides* feces; (d) *Q. u-lucida* feces; (e) incubation of grandisin with digestive enzymes from *Q. u-lucida*.

with the presence of two methoxyl groups at δ 3.86 and 3.84, and one phenolic hydroxyl at δ 6.46. Thus, compound **2b** was determined to be 3-hydroxy-4,5-dimethoxycinnamyl alcohol. This structure was further confirmed by comparison of IR and ^{13}C NMR spectroscopic data with those of the compound previously characterized from roots of *Ferula sinaica* (Ahmed, 1991). Compound **2b** could be formed by 3-*O*-demethylation of **2a** by *N. bipes*, but two possibilities for the formation of **2a** could be envisaged. The first considered it as product of *E*-isoelemicin (**2**) oxidation during the digestive process of *N. bipes*, since there is no detectable amount of **2** remaining in fecal extracts. This type of reaction had been observed with the phenylpropanoid *trans*-anethole after its oral and topical administration in the last instars larvae of *S. litura* and *Trichoplusia ni* (Passreiter et al., 2004). The alternative

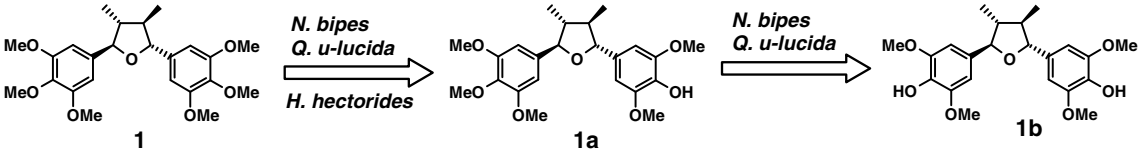

Fig. 2. Biotransformations of (−)-grandisin (**1**) from *P. solmsianum* leaves by *N. bipes*, *Q. u-lucida* and *H. hectorides*.

possibility would involve its release from a glucoside form present in the leaf during the digestive process of *N. bipes* (Fig. 3). Thus, in order to determine whether the glycoside form of **2a** was of natural occurrence, the water-soluble fraction and the hydrolyzed fraction from the debris of water-extracted *P. solmsianum* leaves were analyzed by ^{1}H and ^{13}C NMR spectroscopy. The data of an enriched fraction indicated the presence of phenylpropanoid glycosides D-glucosyl 1-(3,4,5-trimethoxycinnamate) (**3**) and D-glucosyl 1-(3,4,5-trimethoxybenzoate) (**4**), respectively, as compared to previously reported data (Shimomura et al., 1986; Patil et al., 1966). Nevertheless, since there was no evidence for the occurrence of **2a** as its glycosilated form, its formation from oxidation of **2** could be envisaged.

Compound **3a** was identified as 3,4,5-trimethoxycinnamic acid based on interpretation and comparison of H^1 NMR and EIMS data with those previously reported (Lloyd et al., 1976). Compound **3a** could be a hydrolysis product of **3**.

The fecal extracts from *H. hectorides* caterpillars fed with *P. solmsianum* leaves also contained **1a** and **3a**, as observed for fecal extracts from the beetle *N. bipes* (Fig. 1c). Nevertheless, a significant proportion of *E*-isoelemicin (**2**) had remained, relatively to (−)-grandisin (**1**). (−)-Grandisin (**1**) was mono-*O*-demethylated to **1a**, but no di-*O*-demethylation apparently took place. The major observed compounds were **3a** and **4a**, which were isolated and identified, based on analysis of EIMS and H^1 NMR spectrometric data as 3,4,5-trimethoxycinnamic (**3a**) acid and 3,4,5-trimethoxybenzoic acid (**4a**), respectively (Karzhaubekova and Burasheva, 2002). Compound **4a** could be the hydrolysis product of **4** during the digestive process in *H. hectorides*.

The chromatographic profile of fecal extracts of *Q. u-lucida* caterpillars fed with fresh *P. solmsianum* leaves indicated **1b** as the major detectable compound (Rt 17 min) (Fig. 1d). The structure of the compound was determined, based on the retention time and mass spectral data (EI and ESI, *m*/*z* 404 and 405, respectively), as 4,4′-di-*O*-demethylgrandisin (**1b**). Since *Q. u-lucida* larvae were abundant, further investigation on such biotransformation through incubation of **1** with digestive enzymes removed from caterpillar and subsequent LC–MS analysis were carried out, and approximately 90% of conversion to **1b** was observed after 1 h of incubation (Fig. 1e).

In summary, the Lepidoptera *Q. u-lucida* and *H. hectorides*, as well as the Coleoptera *N. bipes*, were capable of biotransforming the tetrahydrofuran (−)-grandisin (**1**) to the corresponding mono- (**1a**) and di-*O*-demethylated (**1b**) phenolic derivatives, when *P. solmsianum* leaves were provided as their sole diet. The demethylation reactions are apparently regiospecific at *para* position, similarly to the demethylation of the furofuran (+)-magnolin by *S. litura* (Miyazawa et al., 1995) and of tetrahydrofuran lignans by *A. niger* (Kasahara et al., 1996a). It was not clear whether the phenylpropanoids had the *meta*-*O*-methyl groups removed in a different fashion from that observed for (−)-grandisin. Despite the lack of data on the toxicity of grandisin on such insects, some preliminary hypotheses can be envisaged. The antifeeding activity of lignans and phenylpropanoids is often reduced by an increase in their polarity, especially through hydroxylation and/or glycosylation. On the other hand, non-polar substituents, such as methoxyl or methylenedioxyphenyl groups, could enhance toxicity (Harmatha and Nawrot, 2002). Nevertheless, arguments for such processes as a mechanism to rendering them less harmful within the insect digestive system are speculative and controversial. For instance, the phenolic compounds produced by demethylation of aromatic methoxyl groups could be expected to be unpalatable or at least be deterrent for insects. On the other hand, the defensive and antioxidant properties of the phenolic compounds could eventually represent a benefit for the herbivorous insects against their predators or to their overall health.

3. Experimental

3.1. General

Silica gel (Merck 230-400 mesh) was used for column chromatography and solvents were redistilled prior to use. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz (Bruker DPX-300, Bruker BioSpin, Rheinstetten Germany), 200 and 50 MHz, (Bruker AC-200) using $CDCl_3$ and CD_3OD (Aldrich, St. Louis, MO, USA) as

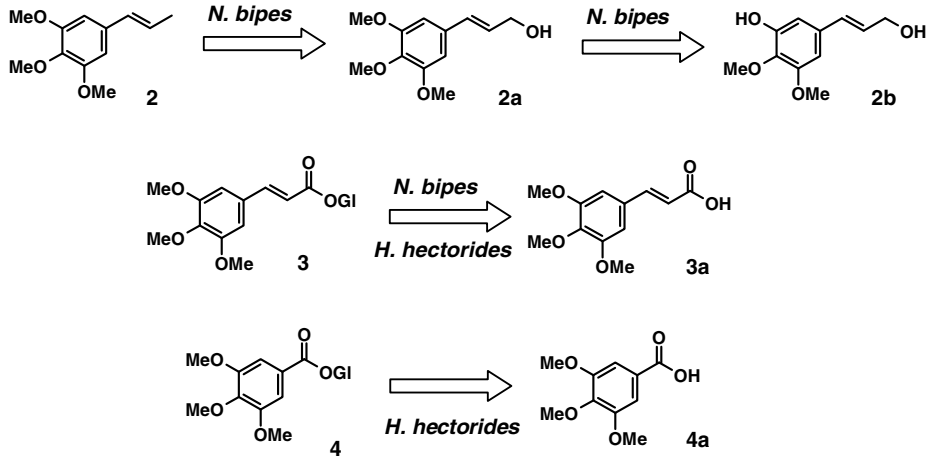

Fig. 3. Proposed biotransformations of phenylpropanoids and/or release of glycosylated acids from *P. solmsianum* leaves by *N. bipes*, *Q. u-lucida* and *H. hectorides*.

solvents. Chemical shifts were reported in δ units (ppm) relative to TMS and coupling constants (*J*) in Hz. IR spectra were obtained on a Perkin Elmer Nicolet 1750 (Palo Alto, CA, USA). EI-MS was measured at 70 eV on a HP 5990/5988A spectrometer. ESI was measured at 4 kV, on an Agilent Technologies, model SL. HPLC analyses of extracts and pure compounds were performed on a HP-1050 instrument using a C18 column (250 × 4.6 mm, 5 μm) from Supelco (Bellefonte, PA, USA) eluted in a gradient mode starting with CH_3CN:H_2O (3:7) for 8 min, raising to 100% of CH_3CN in 37 min, with detection at 254 nm. GC–FID analyses were carried out on a Shimadzu (Tokyo, Japan) 17A instrument equipped with a HP DB-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness, cross-linked 5% phenylmethyl silicone). Temperature gradient: from 100° to 280° at 3° min^{-1} and then held at 300° during 15 min. The flow rate of carrier gas (He) was 1.6 ml min^{-1}.

3.2. Study site and plant material

Field observations were conducted in a garden at Instituto de Química of Universidade de São Paulo (São Paulo, Brazil) from 2000 to 2004. Out of 65 Piperaceae species currently under cultivation, only *P. gaudichaudianum*, *P. regnellii* and *Pothomorphe umbellata* were originally found in this area. Moreover, additional species on this site include *Virola sebifera* and *V. oleifera* (Myristicaceae); *Araucaria angustifolia* (Araucariaceae); *Psidium guayava* (Myrtaceae); and *Cecropia* spp. (Cecropiaceae). *P. solmsianum* was identified by Dr. Elsie F. Guimarães (Jardim Botânico do Rio de Janeiro – Brazil). A voucher specimen (329676, *P. solmsianum*) was deposited at the Herbarium of Instituto de Pesquisas Jardim Botânico do Rio de Janeiro.

3.3. Plant extractions

All dried plant material (40 °C) was ground to a powder (1–2 g) and extracted with CH_2Cl_2:MeOH (2:1, 20 ml × 3), evaporated under reduced pressure and subjected to HPLC and spectrometric analysis. For GC/MS and HPLC analysis extracts 1 and 10 μl, respectively, of a 2 mg ml^{-1} MeOH solution, were injected.

3.4. Isolation of tetrahydrofuran lignans from P. solmsianum

The phenylpropanoid *E*-isolemicin (**2**) and the tetrahydrofuran lignan (−)-grandisin (**1**) were isolated from leaves and inflorescences of *P. solmsianum* as previously reported (Martins et al., 2000, 2003).

3.5. Insect material

The Lepidoptera and Coleoptera species were collected at São Paulo – SP, Campus of Universidade de São Paulo (Brazil) and were identified by Dr. Sérgio Antônio Vanin (Departmento de Zoologia of the Instituto de Biociências, Universidade de São Paulo). Voucher specimens, *N. bipes* (CSR 001), *Q. u-lucida* (CSR005), *H. hectorides* (CSR006) were deposited at the Museu de Zoologia, USP (São Paulo, Brazil) (Vanin et al., 2008).

3.6. Isolation of **1a**, **1b**, **2a**, **2b** and **3a** from feces of N. bipes

Ten individuals of *N. bipes* were maintained in cages for a month under a diet consisting of *P. solmsianum* leaves, with feces collected daily. Dried feces (400 mg, 40 °C for 48 h) were milled and extracted with CH_2Cl_2:MeOH (2:1) three times, which after concentration in vacuum yielded 90 mg of crude extract. The extract was dissolved in EtOAc (70 ml) and extracted with 1 M NaOH (50 ml) three times. The aq. fraction was acidified to pH 2.0 with HCl (1 M) and extracted with EtOAc three times. The combined organic fraction was dried with anhydrous Na_2SO_4 and conc. in vacuum to yield 30 mg of a phenolic fraction. This fraction was subjected to CC and eluted with hexane:EtOAc (3:2), followed by prep. TLC using hexane:EtOAc (3:2, three elutions) to afford the compounds **1a** (1.8 mg), **1b** (1.1 mg), **2a** (6 mg), 2b (1.5 mg) **3a** and (0.9 mg).

3.7. Isolation of **1a**, **3a** and **4a** from feces of H. hectorides

Dried feces (400 mg, 40 °C for 48 h) of *H. hectorides* (*N* = 6) fed with *P. solmsianum* leaves were milled and extracted with CH_2Cl_2:MeOH (2:1) three times (200 ml each) and then concentrated in vacuum to yield 152 mg of crude extract. This extract was dissolved in EtOAc (100 ml) and extracted with 1 M NaOH three times (80 ml each). The aq. solution was acidified to pH 2.0 with HCl (1M) and extracted with EtOAc three times (70 ml). Concentration of EtOAc solution under vacuum afforded a phenolic fraction (80 mg) which was submitted to preparative TLC (hexane:EtOAc, 3:2, four times) to yield **1a** (3.0 mg), **3a** (2.0 mg) and **4a** (1.3 mg).

3.8. Isolation of **1b** from feces of Q. u-lucida

Dried feces (150 mg, 40 °C for 48 h) of *Q. u-lucida* (*N* = 5) fed with *P. solmsianum* leaves were milled and extracted with CH_2Cl_2:MeOH 2:1 three times (100 ml each) and then concentrated in vacuum to yield 40 mg of crude extract. This extract was dissolved in EtOAc (50 ml) and extracted with 1 M aq. NaOH three times (30 ml each). The aq. solution was acidified to pH 2.0 with HCl (1M) and extracted with EtOAc three times (30 ml). The combined EtOAc solution was dried with anhydrous Na_2SO_4, filtered and concentrated under vacuum to afford a phenolic fraction (10 mg) which was submitted to preparative TLC (hexane:EtOAc, 3:2, four times) to afford compound **1b** (1.0 mg).

3.9. Phenylpropanoid glycosides

Dried leaves of *P. solmsianum* (500 g, 40 °C for 48 h) were extracted with 80% EtOH (2 L). The extract was concentrated under vacuum and the residue (35 g) was suspended in H_2O (500 ml) and sequentially extracted with Et_2O (2 × 200 ml) and *n*-BuOH (2 × 200 ml). The *n*-BuOH fraction (4.5 g) obtained after concentration in vacuum was chromatographed on a Sephadex G15 column (Sigma Chemical Co., St. Louis, MO, USA), eluted with a gradient MeOH–H_2O 1:1 to 9:1 yielding five fractions (I–V, 150 ml each). Fraction II was chromatographed on a C18 column, eluted under isocratic condition with MeOH:H_2O (1:4) to yield a fraction containing a mixture of **3** and **4** (80 mg).

3.10. Incubation of (−)-grandisin

The substrate (−)-grandisin (**1**) (50 μg) dissolved in 50 μl of DMSO was incubated with gut fluid removed from two *Q. u-lucida* caterpillars (300 μl) in a 2 ml conical flask containing 500 μl of 0.1 M KPi buffer pH 6.0. After 1 h incubation at 30 °C, the mixture was extracted with EtOAc (3 × 2 ml) and concentrated under vacuum. The extract was subjected to LC–MS and the product corresponding to **1b** was observed.

Compound ***1a***. 4-*O*-demethylgrandisin. $C_{23}H_{30}O_7$. EI-MS *m*/*z* (rel. int.): 418 $[M]^+$ (23), 236 (27), 224 (39) 222 (36), 210 (44), 208 (100), 196 (6), 194 (44) and 182 (7). ^1H and ^{13}C NMR were similar to literature (Barbosa-Filho et al., 1989).

Compound ***1b***. 4,4′-Di-*O*-demethylgrandisin. $C_{22}H_{28}O_7$. EI-MS *m*/*z* (rel. int.): 404 $[M]^+$ (4), 222 (30), 210 (70), 194 (100) and 182 (10). ^1H and ^{13}C NMR were similar to literature (Barbosa-Filho et al., 1989).

Preparation of 4,4′-di-*O*-demethylgrandisin (***1b***): Compound **1b** was prepared as previously reported (Sarkanen and Wallis, 1973).

Compound ***2a***. 3,4,5-Trimethoxycinnamyl alcohol. $C_{12}H_{16}O_4$. EI-MS *m*/*z* (rel. int.): 224 $[M]^+$ (100), 210 (7), 181 (64), 91 (47). ^1H and ^{13}C NMR were similar to literature (Sadik et al., 2003).

Compound ***2b***. 3-Hydroxy-4,5-dimethoxycinnamyl alcohol. $C_{11}H_{14}O_4$. EI-MS *m*/*z* (rel. int.): 210 $[M]^+$ (24), 181 (57), 91 (43), 43 (100). ^1H and ^{13}C NMR were similar to literature (Ahmed, 1991).

Compound ***3a***. 3,4,5-Trimethoxycinnamic acid. $C_{12}H_{14}O_5$. EI-MS *m*/*z* (rel. int.): 238 $[M]^+$ (100), 223 (48) 163 (23) and 181 (13), similar to the data available in the mass spectral libraries (NIST21, NIST107, Wiley 229) and previously reported data (Lloyd et al., 1976).

Compound ***4a***. 3,4,5-Trimethoxybenzoic acid. $C_{10}H_{12}O_5$. EI-MS *m*/*z* (rel. int.): 212 $[M]^+$ (100), 197 (61), 141 (26) and 93 (19), similar to the data available in the mass spectral libraries (NIST21, NIST107, Wiley 229) and previously reported data (Karzhaubekova and Burasheva, 2002).

Acknowledgements

This work was funded by Grants from FAPESP and CNPq. M.J.K. is grateful to CNPq for research fellowship. C.S.R. thanks FAPESP for providing a scholarship.

References

Ahmed, A.A., 1991. Daucanes and other constituents from *Ferula sinaica*. Phytochemistry 30, 1207–01210.

Barbosa-Filho, J.M., Silva, M.S., Yoshida, M., Gottlieb, O.R., 1989. Neolignans from *Licaria aurea*. Phytochemistry 28, 2209–2211.

Benevides, P.J.C., Sartorelli, P., Kato, M.J., 1999. Phenylpropanoids and neolignans from *Piper regnellii*. Phytochemistry 52, 339–343.

Campos, M.P., Filho, V.C., Silva, R.Z., Yunes, R.A., Zacchino, S., Juarez, S., Cruz, R.C.B., Cruz, A.B., 2005. Evaluation of antifungal activity of *Piper solmsianum* C. DC. var. *solmsianum* (Piperaceae). Biol. Pharm. Bull. 28, 1527–1530.

Harmatha, J., Nawrot, J., 2002. Insect feeding deterrent activity of lignans and related phenylpropanoids with a methylenedioxyphenyl (piperonyl) structure moiety. Entomol. Exp. Appl. 104, 51–60.

Karzhaubekova, Z.Z., Burasheva, G.S., 2002. Phytochemical study of *Kalidium caspicum*. Chem. Nat. Compd. 38, 100–101.

Kasahara, H., Miyazawa, M., Kameoka, H., 1996a. *O*-demethylation of 7,7′-epoxylignans by *Aspergillus niger*. Phytochemistry 43, 111–113.

Kasahara, H., Miyazawa, M., Kameoka, H., 1996b. *meta*-*O*-demethylation of (−)-magnofargesin in *Spodoptera litura*. Phytochemistry 43, 1217–1218.

Lanteri, A.A., Guedes, J.C., Parra, J.R.P., 2002. Systematic, morphology and physiology: weevils injurious for roots of citrus in São Paulo state, Brazil. Neotrop. Entomol. 31, 561–569.

Lloyd, R.A., Miller, C.W., Roberts, D.L., Giles, J.A., Dickerson, J.P., Nelson, N.H., Rix, C.E., Ayers, P.H., 1976. Flue-cured tobacco flavor. I. Essence and essential oil components. Tob. Sci. 20, 125–133.

Martins, R.C.C., Latorre, P., Kato, M.J., 2000. Phenylpropanoids and tetrahydrofuran lignans from *Piper solmsianum*. Phytochemistry 55, 843–846.

Martins, R.C.C., Lago, J.H.G., Albuquerque, S., Kato, M.J., 2003. Trypanocidal tetrahydrofuran lignans from inflorescences of *Piper solmsianum*. Phytochemistry 64, 667–670.

Moreira, D., De, L., Guimarães, E.F., Kaplan, M.A.C., 1995. Chemical constituents from *Piper solmsianum* C.. DC. Rev. Bras. Ciênc. Farm. 76, 106–109.

Miyazawa, M., Kasahara, H., Kameoka, H., 1995. Biotransformation of lignans: metabolism of (+)-eudesmin and (+)-magnolin in *Spodoptera litura*. Phytochemistry 39, 1027–1028.

Miyazawa, M., 2001. Biotransformation of lignans and neolignans. Curr. Org. Chem. 5, 975–986.

Miyazawa, M., Ando, H., Okuno, Y., Araki, H., 2004. Biotransformation of isoflavones by *Aspergillus niger* as biocatalyst. J. Mol. Catal. B: Enzym. 27, 91–95.

Nawrot, J., Harmatha, J., 1994. Natural products as antifeedants against stored products insects. Postharvest News Inf. 5/2:17N-21N.

Novotny, V., Miller, S.E., Cizek, L., Leps, J., Janda, M., Basset, Y., Weiblen, G.D., Darrow, K., 2003. Colonising aliens: caterpillars (Lepidoptera) feeding on *Piper adundum* and *P. umbellatum* in rainforests of Papua New Guinea. Ecol. Entomol. 28, 704–716.

Passreiter, M.C., Wilson, J., Andersen, R., Isman, B.M., 2004. Metabolism of thymol and *trans*-anethole in larvae of *Spodoptera litura* and *Trichoplusia ni* (Lepidoptera: Noctuidae). J. Agric. Food Chem. 52, 2549–2551.

Patil, S., Zucker, M., Dimond, A.E., 1966. Biosynthesis of chlorogenic acid in potato roots resistant and susceptible to *Verticillium albo-atrum*. Phytopathology 56, 971–974.

Penz, C.M., Araújo, A.M., 1990. Interaction between Papilio hectorides (Papilionidae) and four host plants (Piperaceae, Rutaceae) in a southern Brazilian population. J. Res. Lepid. 29, 161–171.

Sadik, G., Islam, R., Rahaman, M.M., Khondkar, P., Rashid, M.A., Sarker, S.D., 2003. Antimicrobial and cytotoxic constituents of *Loranthus globosus*. Fitoterapia 74, 308–311.

Sarkanen, K.V., Wallis, A.F., 1973. Oxidative dimerizations of (*E*) and (*Z*)-2,6-dimethoxy-4-propenylphenol. J. Chem. Soc. Perkin Trans. I, 1869–1875.

Shimomura, H., Sashida, Y., Adachi, T., 1986. Phenolic glucosides from *Prunus grayana*. Phytochemistry 26, 249–251.

Singh, R.P., Tomar, S.S., Attri, B.S., Parmar, B.S., Maheshwary, M.L., Mukerjee, S.K., 1976. Search for new *Pyrethrum* synergists in some botanicals. Pyrethrum Post 13, 91–93.

Smeds, A.I., Willfoer, S.M., Pietarinen, S.P., Peltonen-Sainio, P.R., Markku, H.T., 2007. Occurrence of "mammalian" lignans in plant and water sources. Planta 226, 639–646.

Srivastava, S., Gupta, M.M., Prajapati, V., Tripathi, A.K., Kumar, S., 2001. Sesamin a potent antifeedant principle from *Piper mullesua*. Phytother. Res. 15, 70–72.

Vanin, A.S., Ramos, C.S., Guimarães, E.F., Kato, M.J., 2008. Insect feeding preferences on Piperaceae species observed in Sao Paulo city, Brazil. Rev. Bras. Entomol. 52, 72–77.