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Article

Identification of Semiochemicals in Adults and Nymphs of the Stink Bug *Pallantia macunaima* Grazia (Hemiptera: Pentatomidae)

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O conteúdo das glândulas abdominais dorsais em ninfas e das glândulas metatorácicas em machos e fêmeas (10, 20 e 30 dias de idade adulta) foi caracterizado e quantificado para o percevejo *Pallantia macunaima*. O principal componente encontrado nas ninfas e adultos foi o tridecano, com menores quantidades de outros hidrocarbonetos alifáticos, aldeídos, oxo-alcenais e ésteres. Os cinco ínstares apresentaram diferenças significativas nas proporções dos compostos encontrados, principalmente entre aqueles do primeiro ínstar em relação aos demais ínstares. Nenhuma diferença significativa foi detectada na proporção dos compostos das glândulas metatorácicas entre os machos e fêmeas, porém, entre indivíduos de diferentes idades, (*E*)-2-hexenal e acetato de (*E*)-2-decenila diminuiram significativamente de 10 para 20 dias de idade.

The contents of the dorsal abdominal glands in nymphs and the metathoracic glands in adult males and females (10, 20 and 30 days old) were characterized and quantified for the stink bug, *Pallantia macunaima*. The major component for nymphs and adults was tridecane, with lesser amounts of other aliphatic hydrocarbons, aldehydes, oxo-alkenals and esters. The five nymphal instars showed significant differences in the proportions of compounds present, mainly between those of the first instar compared to the dorsal abdominal glands components of later instars. No significant differences were detected in the proportion of metathoracic gland components between the sexes but, between individuals of different ages, (*E*)-2-hexenal and (*E*)-2-decenyl acetate significantly decreased in adults from 10 to 20 days of age.

Keywords: allomone, pheromone, gland, hemiptera, *Pallantia macunaima*

Introduction

Stink bugs (Heteroptera: Pentatomidae) are among the main agricultural pests in the world, and they are increasingly important with the advent of genetically modified crops.[1] The piercing-sucking mode of feeding exhibited by stink bugs is particularly damaging to maturing fruit and seeds, and stink bugs often migrate undetected into maturing crops from wild hosts plants or other crops.[2] *Pallantia macunaima* is one of the important heteropteran pests found in southern Brazil.[1] New methods are needed to minimize or eliminate application of environmentally harmful insecticides used to control this stink bug, as well as other pest species.

Pheromones are potentially useful for monitoring and otherwise managing pest species, and significant progress has been made in the identification of the pheromones of Heteroptera[3] since the first heteropteran pheromone was identified.[4] However, the identification of heteropteran pheromones is complicated by the fact that these so-called "true bugs" characteristically produce large quantities of strong-smelling and irritating defensive chemicals (allomones), which are released when the bugs are disturbed.[5] This mixture of chemical compounds frequently serves as both an alarm pheromone and as an allomone for defense against predators.[5] Heteropteran nymphs produce allomones in dorsal abdominal glands (DAGs), the contents of which are shed along with the exuviae each time the nymph molts, and extraction of exuviae is a convenient means to obtain DAG secretion

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for identification.[6] In some species, the DAG secretions of nymphs act as aggregation pheromones or arrestants; for example, *(E)*-4-oxo-2-decenal is characteristic of first-instar nymphs[6] and serves as an aggregation pheromone.[7,8] In adults, allomones are produced in the metathoracic scent gland (MTG). Identification of MTG secretions has received considerable attention, in part, because these secretions constitute such an obvious defense, and the components are simple compounds produced in comparatively large quantities making them easy to analyze and identify.[5,9] The compositions of stink bug allomones are similar for most species, including normal hydrocarbons, plus saturated and unsaturated aldehydes and esters.[5]

The main purpose of the present work was (*i*) to identify and quantitate the chemical composition of the exuviae for the five nymphal instars and, (*ii*) to identify the chemical components of the MTG secretion for *P. macunaima* adults and, (*iii*) to compare the composition of the secretions from males and females of differing ages (10, 20 and 30 days post-emergence).

Experimental

Insects

A colony of *P. macunaima* was started from bugs provided by Dr. A. R. Panizzi (EMBRAPA Soja, Londrina, Paraná, Brazil).The bugs were reared on soybean seeds (*Glycine max*), green beans (*Phaseolus vulgaris*), peanuts (*Arachis hypogaea*), and glossy privet (*Ligustrum lucidum*) at 26 ± 2 ºC, 70% relative humidity, and a 14:10 h light-dark cycle.

Extraction of dorsal abdominal glands contents

Hexane extracts of the DAGs for the five instars were prepared by extracting exuviae collected ≤ 24 h after ecdysis. Ten exuviae were extracted for each sample of first instars, 8 for second instars, 6 for third instars, 5 for fourth instars, and 3 for fifth instars. Exuviae were extracted for 24 h, after which the hexane extract was transferred to another clean vial and stored in a freezer −20 ºC until analysis. Three extracts were prepared and analyzed for each nymphal instar.

Extraction of metathoracic gland secretion

An adult *P. macunaima* was pinned dorsal-side-up through the prothorax in a Petri dish, and submerged in tap water. The dissection process (using small surgical scissors and sharpened forceps) consisted of removing the wings, cutting the lateral margins of the abdomen anteriorly up to the metathorax, and transversely cutting the anterior margin of the scutellum. The tergal cuticle was pulled back, and the viscera were removed. The scent gland complex, located in the ventral metathoracic region, could then be reached and removed by cutting laterally through the meso- and metathorax, turning the preparation over, and cutting transversely between the meta and prothorax. The gland reservoir, including the lateral accessory glands,[10] was removed, dried with tissue paper, immersed in 200 μL of analytical grade hexane, and stored at −20 ºC until analysis. Five MTG extracts were prepared for each sex and age (10, 20 and 30 days post-emergence).

Chemical analyses

Extracts were analyzed (1 μL of extract) by coupled gas chromatography-mass spectrometry (GC-MS) using a Shimadzu QP-5050A GC-MS operated in the electron impact ionization mode (70 eV), and equipped with a DB-5 capillary column (30 m × 0.25 mm i.d. × 0.25 μm; J & W Scientific, Folsom, California, USA). The GC was operated in the splitless mode, programming the temperature from 50 ºC for 1 min, then increasing at 7 ºC min^{-1} to 250 ºC, and holding at this temperature for 10 min. The National Institute of Standards and Technology (NIST) mass spectral chemical database was used.

Chemical standards

(*E*)-2-Hexenal, (*E*)-2-octenal, (*E*)-2-decenal, (*E,Z*)-2,4-decadienal and (*E*)-2-decen-1-ol were purchased from Acros Organics (Geel, Turnhout, Belgium). Undecane, dodecane, tridecane, tetradecane, pentadecane, 1-tridecene and (*E*)-2-hexenyl acetate were purchased from Aldrich Chemical Company (Milwaukee, Wisconsin, USA). *(E)*-4-oxo-2-Hexenal and *(E)*-4-oxo-2-decenal were gifts from Dr. K. Chauhan, USDA-ARS Beltsville, MD, USA,[11] and Prof. Dr. J. G. Millar, University of California, Riverside, CA, USA,[12] respectively.

(*E*)-2-Decenyl acetate was prepared by allowing a mixture of (*E*)-2-decen-1-ol (1.1 g), acetic anhydride (1.1 g), triethylamine (1.5 mL), and 4-(dimethylamino)pyridine (0.075 g) to stand for 24 h at room temperature. Ether and 2 mol L^{-1} HCl were added, and the organic phase was washed with saturated $NaHCO_3$, dried over $MgSO_4$, and the solvent evaporated in vacuo.[8,9] The resulting product, (*E*)-2-decenyl acetate [*m/z* 198 (M^+, < 1%), 138 (M-60, 4%), 41 (25%), 43 (100%)], was > 94% pure by GC-MS.

Statistical data analysis

Analysis of variance (ANOVA) followed by the Tukey test and variation analysis was used to compare the percentages of compounds present in extracts of exuviae and MTG. In addition, a cluster analysis was performed for the percentages of MTG compounds, and for using a Euclidean distance dissimilarity measure.[13]

Results and Discussion

A maximum of eight compounds were commonly found in the DAG secretions of *P. macunaima* nymphs (Figure 1). Mass spectral data suggested that compounds A and C-H were (*E*)-2-hexenal, (*E*)-2-octenal, undecane, dodecane, (*E*)-2-decenal, 1-tridecene, and tridecane, respectively. These identifications were confirmed by GC coinjection, and mass spectral comparisons with those of authentic standards. A matching mass spectrum for compound B was not found in the GC-MS database, but this compound exhibited characteristic ions at *m/z* (%) 112 (11), 97 (4), 83 (100) and 55 (78) (M^+, $CHOCHCHCOCH_2^+$, $CHOCHCHCO^+$ and $CHOCHCH^+$, respectively) matching the spectrum for (*E*)-4-oxo-2-hexenal.[14] The identity of this compound in *P. macunaima* nymphs as (*E*)-4-oxo-2-hexenal was confirmed by GC coinjection with the authentic standard.[11,12]

The GC trace for the exuvial extract of first-instar nymphs was significantly different from that for second- to fifth-instar nymphs according to analysis of variance, while the composition of exuvial extracts for second- to fifth-instars did not differ significantly (Table 1). Tridecane and (*E*)-4-oxo-2-hexenal are major components in first instars, followed by lesser amounts of 1-tridecene and (*E*)-2-decenal. The major component from second instars is tridecane, followed by significant amounts of 1-tridecene, (*E*)-2-hexenal, (*E*)-2-decenal and (*E*)-4-oxo-2-hexenal, plus traces of undecane, dodecane and (*E*)-2-octenal.

Four compounds varied in relative abundances among the exuvial extracts analyzed for nymphs (Tables 1 and 2): dodecane, (*E*)-2-hexenal, (*E*)-2-decenal and (*E*)-4-oxo-2-

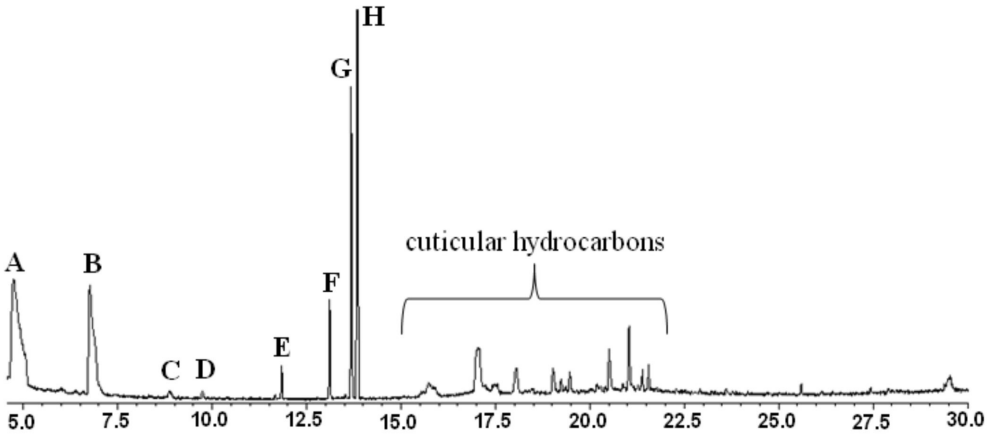

Figure 1. Gas chromatogram of exuviae extract from a fifth-instar *P. macunaima* nymph. Compounds: A: (*E*)-2-hexenal, B: (*E*)-4-oxo-2-hexenal, C: (*E*)-2-octenal, D: undecane, E: dodecane, F: (*E*)-2-decenal, G: 1-tridecene, H: tridecane.

Table 1. Relative abundances (%) of compounds present in *P. macunaima* exuvial extracts of first- to fifth-instar nymphs (mean ± SD) (n = 3)

		Pallantia macunaima				
Group	Compound	1st	2nd	3rd	4th	5th
Alkane	Undecane	0.0 ± 0.0a	0.1 ± 0.0a	0.2 ± 0.1a	0.3 ± 0.0a	0.7 ± 0.5a
	Dodecane*	0.0 ± 0.0a	0.8 ± 0.2b	1.0 ± 0.1b	1.1 ± 0.2b	1.2 ± 0.1b
	Tridecane	30.1 ± 2.0a	34.6 ± 2.5a	42.1 ± 4.3a	43.6 ± 3.4a	35.4 ± 8.3a
Alkene	1-Tridecene	10.6 ± 1.0a	13.3 ± 1.2a	15.1 ± 0.1a	13.6 ± 1.6a	11.3 ± 2.3a
Aldehyde	(*E*)-2-Hexenal*	0.0 ± 0.0a	19.6 ± 1.9b	20.8 ± 0.6b	25.2 ± 2.2bc	33.6 ± 6.8c
	(*E*)-2-Octenal	0.0 ± 0.0a	0.7 ± 0.1a	0.4 ± 0.1a	0.5 ± 0.1a	1.4 ± 1.1a
	(*E*)-2-Decenal*	27.4 ± 1.5a	12.1 ± 1.0b	7.2 ± 0.6b	7.2 ± 2.0b	8.0 ± 3.3b
Oxo-Alkenal	(*E*)-4-Oxo-2-hexenal*	31.9 ± 0.8a	18.7 ± 2.4b	13.2 ± 3.5b	8.6 ± 0.8b	8.3 ± 7.5b

*Compounds that showed significant differences between the instars. Tukey test ($p > 0.05$).

Table 2. Compounds present in exuvial extracts of *P. macunaima* nymphs, and variation analysis of the percent differences with age

Group	Compound	Analysis[a]	Trend	Change[b]	%[c]
Alkane	Undecane	ns	⇧		0.2
	Dodecane	**	⇧		0.8
	Tridecane	ns	⇧		37.2
Alhene	1-Tridecane	ns	⇨		12.8
Aldehyde	(*E*)-2-Hexenal	**	⇧		19.9
	(*E*)-2-Octenal	ns	⇧		0.6
	(*E*)-2-Decenal	**	⇩		12.4
Oxo-alkenal	(*E*)-4-Oxo-2-hexenal	**	⇩		16.2

[a]ns = not significant; **statistical tests indicates difference between the mean; [b]line formed by means of the treatments (first- to fifth-instars); [c]average of all treatments.

hexenal, with the first two compounds increasing and the last two decreasing in abundance in older nymphs.

The DAG secretion of first-instar nymphs of some, but not all, pentatomid species previously analyzed contain (*E*)-4-oxo-2-decenal, which is totally absent in the secretions of later instars.[6] This first-instar characteristic compound mediates aggregation.[7,8] Interestingly, (*E*)-4-oxo-2-decenal is not produced by first-instars of *P. macunaima* (analyzed using a DB-WAX® polar column; data not shown), yet the data presented herein show that the composition of the DAG secretion differs significantly from that of later instars. The distinguishing feature of the DAG secretion from first-instar *P. macunaima* nymphs is the relatively much greater abundance of (*E*)-2-decenal and (*E*)-4-oxo-2-hexenal (Tables 1 and 2). Therefore, it is tempting to speculate that these compounds serve as an aggregation pheromone for first-instars of *P. macunaima*, which also form tight clusters for much of the duration of this stage, but proof of such a behavioral role awaits further research.

The metathoracic scent gland (MTG) of *P. macunaima* adults is, as in other stink bugs, well developed with an orange colored reservoir that is easily recognized upon dissection. MTG extracts of males and females of this species were typical of other Pentatomidae.[3] In addition to the same compounds found in *P. macunaima* nymphs, adults also produced (*E*)-2-hexenyl acetate, (*E*)-2-decenyl acetate, (*E*,*Z*)-2,4-decadienal, tetradecane and pentadecane (Figure 2). These MTG secretory components exhibited mass spectra matching the spectra of the aforementioned compounds in the NIST computer database, and were

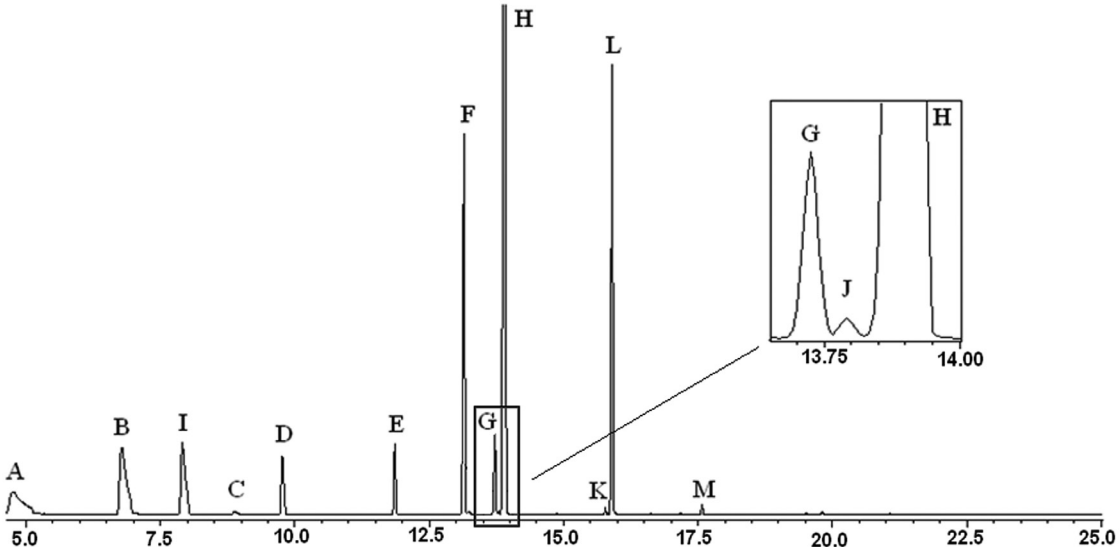

Figure 2. Gas chromatogram of a metathoracic gland extract of a *P. macunaima* adult female 10 days-old. Compounds: A: (*E*)-2-hexenal, B: (*E*)-4-oxo-2-hexenal, C: (*E*)-2-octenal, D: undecane, E: dodecane, F: (*E*)-2-decenal, G: 1-tridecene, H: tridecane, I: (*E*)-2-hexenyl acetate, J: (*E*,*Z*)-2,4-decadienal, K: tetradecane, L: (*E*)-2-decenyl acetate, M: pentadecane.

confirmed by GC coinjection with synthetic standards. MTG extracts from adult males and females of the same age were qualitatively and quantitatively similar, with tridecane being the most abundant constituent of the blend (Table 3). However, we observed significant differences in the proportions of two compounds in the comparisons between the different age groups; namely, (*E*)-2-hexenal and (*E*)-2-decenyl acetate, which each significantly decreased in adults from 10 to 20 days old (Table 4). There were no significant differences in the proportions of MTG compounds from 20 and 30 days of age, as shown in the dendrogram of the cluster analysis (Figure 3).

Table 3. Relative abundances (%) of compounds found in the metathoracic gland (MTG) extracts of *P. macunaima* adults (mean ± SD) (n = 5). Values in % by MTG

Group	Compound*	10 days	20 days	30 days
Alkane	Undecane	2.2 ± 0.5a	1.8 ± 0.3a	2.0 ± 0.8a
	Dodecane	1.6 ± 0.3a	1.7 ± 0.4a	2.1 ± 0.7a
	Tridecane	56.8 ± 8.9a	58.3 ± 11.4a	62.7 ± 6.3a
	Tetradecane	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a
	Pentadecane	0.2 ± 0.1a	0.2 ± 0.1a	0.3 ± 0.1a
Alkene	1-Tridecene	2.0 ± 0.2a	1.7 ± 0.7a	1.9 ± 0.9a
Aldehyde	(*E*)-2-Hexenal*	4.3 ± 2.1a	1.3 ± 0.6b	1.3 ± 0.9b
	(*E*)-2-Octenal	0.2 ± 0.1a	0.3 ± 0.1a	0.3 ± 0.1a
	(*E*)-2-Decenal	17.9 ± 6.5a	21.7 ± 4.6a	17.9 ± 3.3a
	(*E*,*Z*)-2,4-Decadienal	0.2 ± 0.1a	0.2 ± 0.1a	0.3 ± 0.2a
Ester	(*E*)-2-Hexenyl acetate	1.5a ± 1.8a	0.3 ± 0.2a	0.2 ± 0.1a
	(*E*)-2-Decenyl acetate*	4.7 ± 3.0a	1.5 ± 0.7b	1.2 ± 0.8b
Oxo-alkenal	(*E*)-4-Oxo-2-hexenal	9.9 ± 2.5a	12.7 ± 8.1a	11.3 ± 5.3a

*Compounds that showed significant differences with age. Tukey test ($p > 0.05$).

Table 4. Relative abundances (%) of compounds present in MTG extracts of *P. macunaima* adults 10, 20 and 30 days old, and variation analysis of the percent differences with age

Group	Compound	Analysis[a]	Trend	Change[b]	%[c]
Alkane	Undecane	ns	⇨		2
	Dodecane	ns	⇧		1.8
	Tridecane	ns	⇧		59.3
	Tetradecane	ns	⇨		0.1
	Pentadecane	ns	⇨		0.2
Alkene	1-Tridecene	ns	⇨		1.9
Aldehyde	2-Hexenal	**	⇩		2.3
	2-Octenal	ns	⇨		0.3
	2-Decenal	ns	⇨		19.2
	2,4-Decadienal	ns	⇨		0.2
Ester	(*E*)-2-Hexenyl acetate	ns	⇩		0.7
	(*E*)-2-Decenyl acetate	**	⇩		2.5
Oxo-alkenal	4-Oxo-2-hexenal	ns	⇧		11.3

[a]ns = not significant; **statistics test indicates difference between the mean; [b]line formed by means of the treatments (10, 20 and 30 days post-emergence adulthood); [c]average of all treatments.

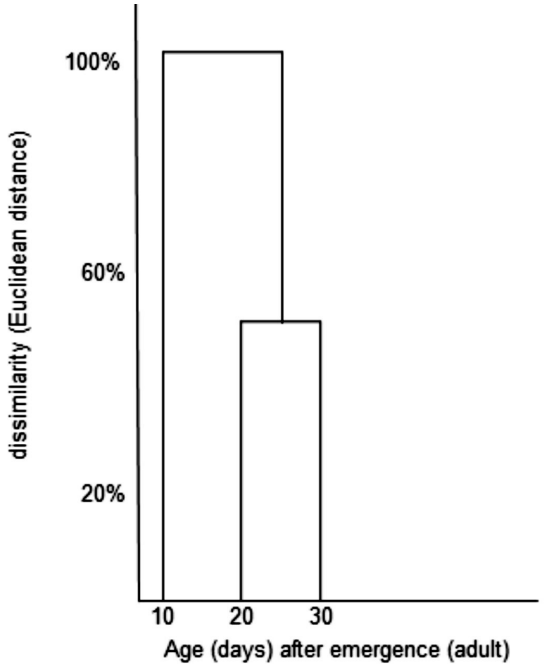

Figure 3. Dendrogram resulting from the analysis of different ages after emergence to the adult stage of *P. macunaima* based on the percentage of the thirteen compounds present in the MTG (X-axis) with the Euclidean distance as similarity measurement (Y-axis). There are two distinct groups; the first group consists of 10-days old adults (males and females), and the second group is a cluster of 20-days and 30-days old adults.

Conclusions

Attractant pheromones are produced in and released from the MTGs of some Heteroptera,[3] probably from the lateral accessory glands attached to the MTG reservoir.[15-17] MTG-derived attractant pheromones are either produced by males (in some Lygaeidae and Alydidae)[15,16,18] or females (Miridae, and some Alydidae),[17,19-23] yet allomones are also stored in and released from the MTG reservoir in these species.[16,17,24] However, no sexual dimorphism was observed for the MTG secretions of *P. macunaima* adults. The reduction of (*E*)-2-decenyl acetate detected as a function of the age of *Pallantia* adults is consistent with the hypothesis that aliphatic aldehydes in the MTG reservoir are derived from the alcohol moiety of the corresponding esters in the lateral accessory glands of the MTG complex,[10,25] although the decrease of (*E*)-2-hexenal with age is inconsistent with this hypothesis. It is possible the differences in MTG chemistry are indicative of the age of bugs, and that this is possibly of ecological relevance, but requires further behavioral experimentation to demonstrate but, in any case, it seems unlikely that the MTG secretion in *P. macunaima* is involved in sex or aggregation pheromone production. Rather, the presumed attractant pheromone of *P. macunaima* is probably produced elsewhere, such as the abdominal sternum of males,[26] as in other pentatomids.[3] Research is currently underway to isolate and identify the attractant pheromone of *P. macunaima*.

Supplementary Information

Mass spectra from all the identified compounds are available free of charge at http://jbcs.sbq.org.br, as a PDF file.

Acknowledgments

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