Acta Farm. Bonaerense **24** (4): 543-5 (2005)
Recibido el 24 de enero de 2005
Aceptado el 22 de mayo de 2005

2,3,4,6-Tetra-*O*-(3-nitropropanoyl)-*O*-β-D-glucopyranoside, a New Antimicrobial from the Roots of *Heteropteris aphrodisiaca*

Walter Antônio Roman JÚNIOR [1], Mara Lane Carvalho CARDOSO [2], Wagner VILEGAS [3]
Celso Vataru NAKAMURA [4], Benedito Prado Dias FILHO [4] and João Carlos Palazzo de MELLO [1,4] *

[1] *Programa de Pós-Graduação em Ciências Farmacêuticas, UNESP, Faculdade de Ciências Farmacêuticas, BR-14801-970, Araraquara, São Paulo, SP, Brazil*
[2] *Departamento de Farmácia e Farmacologia, Universidade Estadual de Maringá Av. Colombo, 5790, BR-87020-900, Maringá, PR, Brazil*
[3] *Instituto de Química, UNESP, BR-14801-970, Araraquara, São Paulo, SP, Brazil*
[4] *Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Estadual de Maringá, Av. Colombo, 5790, BR-87020-900, Maringá, PR, Brazil*

SUMMARY. A new 2,3,4,6-tetra-*O*-(3-nitropropanoyl)-*O*-β-D-glucopyranoside anomer was isolated from the roots of *Heteropteris aphrodisiaca* and characterized by spectroscopic methods. Activity against *Staphylococcus aureus, Bacillus subtilis, Candida albicans, C. parapsilosis, C. krusei,* and *C. tropicalis* was demonstrated.

RESUMEN. "2,3,4,6,-Tetra-*O*-(3-nitropropanoil)-*O*-β-D-glucopiranósido, Un Nuevo Antimicrobiano de las Raíces de *Heteropteris aphrodisiaca"*. Un nuevo 2,3,4,6-tetra-*O*-(3-nitropropanoila)-*O*-β-D-glucopiranósido anomérico fue aislado de las raíces de *Heteropteris aphrodisiaca* y su estructura determinada por métodos espectroscópicos. Su actividad frente a los microorganismos *Staphylococcus aureus, Bacillus subtilis, Candida albicans, C. parapsilosis, C. krusei* y *C. tropicalis* fue demostrada.

INTRODUCTION

The herb called "nó-de-cachorro", *Heteropteris aphrodisiaca* O. Mach., family Malpighiaceae Juss., subgenus *Anosepalis*, section *Microprosopis* [1], grows in the Brazilian Cerrado (central highlands savanna), specifically in the state of Mato Grosso. This region has distinct rainy and dry seasons; the rainy season lasts from November through February.

Nitro compounds are secondary metabolites restricted to the plant families Malpighiaceae, Leguminosae (Papilonoidae) and Aristolochiaceae [2,3]. They have been proposed previously as chemotaxonomic markers, including for taxa of the genus *Heteropteris* [2]. Of these nitro compounds, Finnegan and Stephani [2] determined the structure of hiptagin (**1**) from *Hiptage madablota* Geartn. (Malpighiaceae). After screening 124 Argentinean species of *Heteropteris* from 98 genera, Stermitz and collaborators [3] found **1** in *Heteropteris angustifolia* Gris. The species *H. aphrodisiaca* has not yet been studied from the chemical point of view. Galvão [4] found through phytochemical screening of the roots of *H. aphrodisiaca* that this plant tested positive for polyphenols, phenols, saponins (foam) and alkaloids. The presence of nitro compounds in plant materials is of interest because of their known toxicity to livestock, insects and other animals. Their toxic effects have been reported to be a consequence of their conversion to 3-nitropropanoic acid (3NPA), a respiratory toxin that inhibits mitochondrial enzymes [5], as in the case of miserotoxin (**2**) [6].

We report here the isolation and characterization of a new nitro compound, 2,3,4,6-tetra-*O*-(3-nitropropanoyl)-*O*-β-D-glucopyranoside (**3**), from the roots of *H. aphrodisiaca*. Antimicrobial activity of this nitro compound was also demonstrated.

KEY WORDS: *Heteropteris aphrodisiaca,* Nitrocompound, Malpighiaceae, 2,3,4,6-tetra-*O*-(3-nitropropanoyl)-*O*-β-D-glucopyranoside.
PALABRAS CLAVE: Heteropteris aphrodisiaca, Nitrocompuesto, Malpighiaceae, 2,3,4,6-tetra-*O*-(3-nitropropanoil)-*O*-β-D-glucopiranósido.

* Author to whom correspondence should be addressed. *E-mail:* mello@uem.br

MATERIAL AND METHODS

Plant material

Roots of *Heteropteris aphrodisiaca* O. Mach. were collected in October 2000 at Santo Antônio do Leverger (30°28'31"S and 51°35'25"W; Garmin v. 2.24), Mato Grosso State, Brazil, and identified by Prof. Dr. Miramy Macedo (Universidade Federal de Mato Grosso, UFMT). A voucher specimen (UFMT-22181) was deposited at the UFMT Central Herbarium in Cuiabá, Mato Grosso.

Isolation of the nitro compound 3

Roots (500 g) of *H. aphrodisiaca* were macerated with acetone for 1 week at room temperature (BST 0402). The extract obtained was filtered and the solvent evaporated under vacuum, yielding the crude extract (13 g). A portion of the extract (10 g) was chromatographed on silica gel by vacuum liquid chromatography (VLC) using toluene and then $CHCl_3$/MeOH (97:3; 19:1; 9:1; 17:3; 1:1) as eluent to yield 6 fractions. Fractions (200 ml) were collected and checked by thin layer chromatography (TLC) [Si gel 60 F_{254} plates, solvent systems: n-BuOH:$CHCl_3$:MeOH (55:40:5)]. Fraction 4 (1.0 g) crystallized with methanol:water to yield pure 2,3,4,6-tetra-*O*-(3-nitropropanoyl)-*O*-β-D-glucopyranoside (200 mg) (**3**).

Antimicrobial evaluation

The antimicrobial activity was evaluated against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6623), *Pseudomonas aeruginosa* (ATCC 15442), *Candida albicans, C. parapsilosis, C. krusei,* and *C. tropicalis.* All the *Candida* species were isolated from vaginal mucosa of patients. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)/ Minimal Fungicidal Concentration (MFC) were determined according to the National Committee for Clinical Laboratory Standards [7,8].

RESULTS AND DISCUSSION

Compound **3** was isolated as a white solid, mp. 111-113 °C. The IR spectrum showed intense bands corresponding to hydroxyl (2299 cm^{-1}), ester carbonyl (1747 cm^{-1}) and nitro (1553 and 1385 cm^{-1}) groups. These data were in agreement with the structure of an aliphatic nitro compound [6].

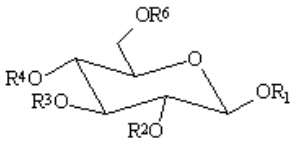
The ESMS (positive mode) mass spectrum gave the pseudo-molecular ions for $[M+Na]^+$ at *m/z* 607 and $[M+NH4]^+$ at *m/z* 602, corresponding to the molecular formula $C_{18}H_{24}O_{18}N_4$. A fragment ion at *m/z* 488 corresponded to the loss of nitropropionic acid $[M-119+Na]^+$. Subsequent loss of HNO_2 from the ion at *m/z* 488 produced the fragment [M-119-47+Na]+ at *m/z* 441. The ion at *m/z* 127 corresponded to the adduct between nitropropionic acid and ammonium $[C_3H_5NO_4+NH_4]^+$.

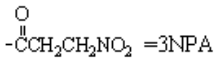
1D- and 2D-NMR analyses led to the data show in Table 1. HMQC showed all of the direct correlations between ^1H and ^{13}C, whereas the HMBC experiment showed all of the long-range couplings between ^1H and ^{13}C. 1D-TOCSY experiments irradiating at the signal at δ 5.82 led to the spin system (H1-H6) characteristic of the glucose unit that was confirmed with the correlations obtained from the $^1H/^1H$ COSY spectrum [9]. The β-configuration of the glucose unit was established by the coupling constant J_{H1-H2}=8.1 Hz [2,10]. In the HMBC spectrum, the signals corresponding to H2, H3, H4, and H6 showed correlations with the carbonyl signals of the corresponding nitropropanoyl moieties. The more deshielded signal at δ 5.82 (H1) did not show a correlation to a carbonyl group, thus indicating a free OH group at C1 of the glucose unit. Therefore, **3** could be identified as the 2,3,4,6-tetra-*O*-(3-nitropropanoyl)-*O*-β-D-glucopyranoside. The two previously isolated 2,3,4,6-tetranitropropanoylglucosides [10,11] had the α-configuration. Alkaline hydrolysis of **3** yielded glucose, checked by TLC with authentic standard [11].

Position	^1H	^{13}C
1''	5.82 d (8.1)	91.87
2''	4.78 m	73.10
3''	4.05 m	71.89
4''	4.83 dd (9.3, 6.9)	70.92
5''	3.92 ddd (9.3, 9.5, 5.6)	70.48
6''	4.08 d (4.5)	62.74
1'	4.80 m	70.10, 70.34, 70.40*
2'	2.85, 3.05, 3.08, 2.97 m	30.85, 30.74, 30.65, 30.65
3'	-	169.20, 169.58, 169.83, 170.23

Table 1. ^1H and ^{13}C data for substance **3** (^1H=300 MHz; ^{13}C=75 MHz; DMSO-d_6). coupling constants in Hz in parentheses; * double intensity.

The isolation of the nitro compound (**3**) from the roots of *Heteropteris aphrodisiaca* O. Mach. confirms the presence of this class of compound in the genus. The occurrence of this compound reinforces the hypothesis that these compounds can be used as taxonomic markers for the genus *Heteropteris.*





1	R^3=H	R^1=R^2=R^4=R^6=3NPA
2	R^1= -$COCH_2CH_2CH_2NO_2$	R^2=R^3=R^4=R^6=3NPA
3	R^1=H	R^2=R^3=R^4=R^6=3NPA

The antimicrobial activity of **3** was determined by microdilution techniques in Mueller-Hinton broth against Gram-positive and Gram-negative bacteria, and Sabouraud dextrose broth for *Candida albicans, C. parapsilosis, C. krusei* and *C. tropicalis.* The minimal inhibitory concentration (MIC) was 125 and 250 µg/ml and the minimal bactericidal concentration (MBC) was 250 and 500 µg/ml against *B. subtilis* and *S. aureus*, respectively. The antifungal activity was stronger than the antibacterial activity, as demonstrated by the MIC. The latter was 125 µg/ml and the minimal fungicidal concentration (MFC) was 250 µg/ml against all *Candida* species. This activity has not been previously described, and this is the first report for *H. aphrodisiaca*. Previous reports were evaluated with isolated nitrocompound by Roman Jr [12] for antibacterial, antifungal and antiviral activity. De Melo [13] demonstrated the antiviral activity of the nitrocompound from the roots of *H. aphrodisiaca*.

Acknowledgements. We thank Laboratórios Biosintética Ltda, Brazil, for research fellowships to W.A. Roman Júnior and for financial support. The present paper is part of the Master of Science Thesis of W.A. Roman Júnior.

REFERENCES

1. Machado, O.X.B. (1948) *Rodriguésia* **11/12**: 113-9.
2. Finnegan, R.A. & R.A. Stephani (1968) *J. Pharm. Sci.* **57**: 353-4.
3. Stermitz, F.R., O. Hnatyszyn, A.L. Bandoni, R.V.D. Rondina & J.D. Coussio (1975) *Phytochemistry* **14**: 1341-5.
4. Galvão, S. M. P. (1997) "Estudo farmacológico e toxicológico de *Heteropteris aphrodisiaca* O. Mach. - Malpighiaceae (nó-de-cachorro) em roedores jovens e idosos". Dissertação (Mestrado), UNIFESP, Universidade Federal de São Paulo, São Paulo, f.107.
5. Anderson, R.C., W. Majak, M.A. Rasmussen, M.J. Allison (1998) "*Detoxification potential of a new species of ruminal bacteria that metabolize nitrate and naturally occurring nitrotoxins*", en "Toxic Plants and Other Natural Toxicants" (T. Garland, A.C. Barr, eds.), New York, CAB International, págs. 144-8.
6. Stermitz, F.R., F.A. Norris & M.C. Williams (1969) *J. Am. Chem. Soc.* **91**: 4599-600.
7. National Committee for Clinical Laboratory Standards (2000) "*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*", Approved Standard M7-A5, vol. 20, #2. National Committee for Clinical Laboratory Standards, Wayne, PA; 5th ed.
8. National Committee for Clinical Laboratory Standards (2002) "*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*", Approved Standard M27-A2, vol.22, #15. National Committee for Clinical Laboratory Standards, Wayne, PA; 2th ed.
9. Benn, M., Y. Bai & W. Majak (1995) *Phytochemistry* **40**: 1629-31.
10. Garcez, S.W., F.R. Garcez, N.K. Honda & A.J.R. da Silva (1989) *Phytochemistry* **28**: 1251-2.
11. Majak, W., M. Benn, D. McEwan, & M.A. Pass (1992) *Phytochemistry* **31**: 2393-5.
12. Roman Jr., W. A., C.V. Nakamura, B.P. Dias Filho, T.U. Nakamura, R.E.C. Linhares, J.C.P de Mello (2001) Atividade antibacteriana, antifúngica e antiviral de nitrocomposto isolado de *Heteropteris aphrodisiaca*. en "Anais do III Simpósio Brasileiro de Farmacognosia", Curitiba, PR, Brasil. pág. OA-3.
13. De Melo, F.L. (2004) "Avaliação da atividade antiviral de compostos isolados de *Heteropteris aphrodisiaca* O. Mach.". Dissertação (Mestrado), Universidade Estadual de Londrina, Londrina, Paraná, f. 47