

# I3-naringenin-II8–4'-OMe-eriodictyol: a New Potential Analgesic Agent Isolated from *Rheedia gardneriana* Leaves

Valdir Cechinel Filho<sup>a,\*</sup>, Karina L. da Silva<sup>a</sup>, Márcia M. de Souza<sup>a</sup>, Ana E. Oliveira<sup>a</sup>, Rosendo A. Yunes<sup>b</sup>, Cláudio L. Guimarães<sup>c</sup>, Luiz G. Verdi<sup>c</sup>, Edesio L. Simionatto<sup>c</sup>, and Franco Delle Monache<sup>d</sup>

<sup>a</sup> Núcleo de Investigações Químico-Farmacêuticas (NIOFAR) CCS, Universidade do Vale do Itajaí (UNIVALI), 88302-202, Itajaí, SC, Brazil

Fax: 00 55 47 3417601. E-mail: [cechinel@mbx1.univali.br](mailto:cechinel@mbx1.univali.br)

<sup>b</sup> Departamento de Química, Universidade Federal de Santa Catarina (UFSC), 88049-900, Florianópolis, SC, Brazil

<sup>c</sup> Departamento de Ciências Naturais, Universidade Regional de Blumenau (FURB), Blumenau, SC, Brazil

<sup>d</sup> Centro Chimica Recettori, CNR, Rome, Italy

\* Author for correspondence and reprint requests

Z. Naturforsch. **55c**, 820–823 (2000); received March 20/May 3, 2000

*Rheedia gardneriana*, GB2a Derivative, Analgesia

This paper describes the isolation, identification and analgesic activity of a new biflavonoid from *Rheedia gardneriana* leaves, which correspond to I3-naringenin-II8-4'-OMe-eriodictyol (GB-2a-II-4'-OMe) (**1**), with a methoxyl group in position 4 of ring-II. Its structure was determined by spectroscopic data and confirmed by an alkaline hydrolysis. Its analgesic effect was evaluated in a writhing test and a formalin test in mice. It was found that this compound exhibits potent and dose-related analgesic action in both experimental models, with ID<sub>50</sub>'s values of 4.5 µmol/kg against the writhing test and 8.2 and 6.8 µmol/kg against the first and second phase of the formalin test, respectively. It was several times more potent than some well-known analgesic drugs used as reference.

## Introduction

We have previously demonstrated that some biflavonoids extracted from *Rheedia gardneriana* leaves, a Brazilian medicinal plant, exhibit analgesic effects in mice. Such compounds, denoted volkensiflavone, I3-naringenin-II8-eriodictyol (GB-2a), fukugetin and fukugiside, were isolated from the ethyl acetate fraction, and caused significant analgesic action in relation to the second phase (inflammatory pain) of the formalin test (Luzzi *et al.*, 1997). In a recent study, we have also shown that another biflavonoid isolated from *Clusia columnaris*, known as GB1a, presented potent and dose-related analgesic activity when evaluated in different models of pain in mice (Bittar *et al.*, 2000).

In this study we have extended our previous findings with the *R. gardneriana* leaves, with the isolation of a new biflavonoid, which was analysed as analgesic or antinociceptive on chemical and thermal models of nociception in mice. Furthermore, the results of some reference drugs were included for the purpose of comparison.

## Material and Methods

### Plant material

The leaves of *R. gardneriana* Pl. Tr. (Guttiferae) were collected from a population growing on the outskirts of FURB, in Blumenau city, in December, 1995. The plant was identified by Prof. Marcos Sobral (Departamento de Botânica, Universidade Federal do Rio Grande do Sul, Porto Alegre) and the vouchers were deposited in Dr. Roberto Miguel Klein Herbarium (Departamento de Ciências Naturais, FURB, Blumenau) under numbers 534 to 540.

### Isolation and identification of biflavonoids

Air dried leaves (150 g) of *R. gardneriana* were powdered and macerated with 50% ethanol (1.2 l) at room temperature for approximately 15 days. After solvent removal, the extract was successively partitioned with hexane, chloroform, ethyl acetate and butanol, respectively.

Ethyl acetate fraction (3.75 g) was chromatographed on a silica gel column eluted with

0939–5075/2000/0900–0820 \$ 06.00 © 2000 Verlag der Zeitschrift für Naturforschung, Tübingen · [www.znaturforsch.com](http://www.znaturforsch.com) · D



Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung“) beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition "no derivative works"). This is to allow reuse in the area of future scientific usage.

CHCl<sub>3</sub>:MeOH gradient, giving four biflavonoids: volkensiflavone (55 mg), GB2a (**2**) (141 mg), fukugetin (810 mg) and fukugiside (100 mg) as previously described (Luzzi *et al.*, 1997). In the course of the experimental procedure, a minor fraction (F1, 60 mg) was also obtained, which gave positive test with FeCl<sub>3</sub> reagent in TLC, indicating the presence of a different phenolic compound. The R<sub>f</sub> value was different from those biflavonoids cited earlier. F1 was then rechromatographed as in the previous case, giving a pure yellow solid (25 mg, mp= 230–3 °C) which was identified as I3-naringenin-II8-4'-OMe-eriodictyol (GB2a-II-4'-OMe) (**1**) on the basis of its spectroscopic data (<sup>1</sup>H- and <sup>13</sup>C-NMR) in comparison with GB2a (**2**) (Table I).

### Pharmacological analysis

#### Writhing test

Male Swiss mice (25–30 g) were used. The abdominal constriction induced by intraperitoneal injection of acetic acid (0.6%), was carried out according to the procedures described previously (Collier *et al.*, 1968; Souza *et al.*, 1998) with minor modifications. The animals were pretreated with compound **1** intraperitoneally (1.75 to 17.5 µmol/kg) 30 min before the acid acetic injection. Control animals received a similar volume of 0.9% NaCl (10 ml.kg<sup>-1</sup>, i.p.). All experiments were carried out at 23 ± 2 °C. After challenge, pairs of mice were placed in separate boxes and the number of abdominal constrictions of the abdominal muscles together with a stretching, were cumulatively counted over a period of 20 min. Antinociceptive activity was expressed as the reduction of the number of abdominal contractions between control animals and mice pretreated with the compound studied.

#### Formalin-induced pain

The procedure used was essentially similar to that previously described (Hunskar *et al.*, 1985; Willain Filho *et al.*, 1997). Animals (25–30 g) from the same strain were slightly anaesthetized with ether, except when used to analyse the first phase of formalin-induced pain, and 20 µl of 2.5% (0.92% formaldehyde) made up PBS (phosphate buffered solution, containing: NaCl 137 mM; KCl

2.7 mM and phosphate buffer 10 mM) was injected s.c. under the plantar surface of the left hindpaw with a Hamilton syringe. Animals were acclimatized to the laboratory for at least 24h before experiments. Two mice (control and treated) were simultaneously observed from 0 up to 30 min following formalin injection. The initial nociceptive scores normally peaked after 5 min (first phase, representing the neurogenic pain), and 15–30 min after formalin injection (second phase, representing the inflammatory pain) (Hunskar *et al.*, 1985). Animals were treated with saline 0.9% (10 ml/kg, i.p.) or with compound **1** (1.75 to 17.5 µmol/kg) 60 min before formalin injection. After intraplantar irritant application, the animals were immediately placed into a glass cylinder (20 cm diameter). The time spent by animals licking or biting the injected paw was timed with a chronometer and was considered indicative of pain.

#### Statistical analysis

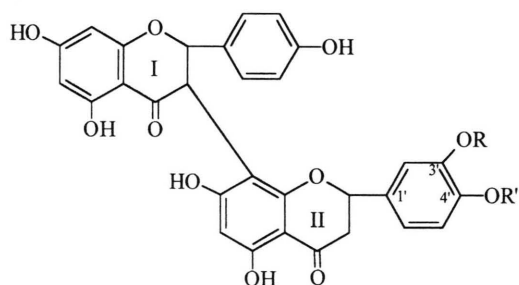
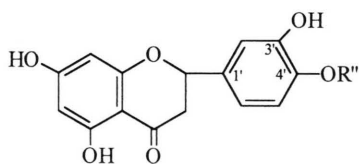
The results are presented as mean ± s.e.m., and the statistical significance between the groups was analysed by means of an analysis of variance followed by Dunnett's multiple comparison test. *P* values less than 0.05 were considered as indicative of significance. The ID<sub>50</sub> values (the dose of the compound that reduced responses by 50% in relation to control values) were estimated by graphical interpolation from individual experiments. ID<sub>50</sub>'s are presented as mean values and 95% confidence interval. MI is the maximum inhibition at higher dose used.

### Results and Discussion

In our previous study with the *R. gardneriana* leaves, we isolated four biflavonoids with analgesic properties (Luzzi *et al.*, 1997). However, we detected other minor phenolic compound present in the ethyl acetate fraction, which gave positive test with FeCl<sub>3</sub> reagent. Considering that the subfraction containing this compound exhibited antinociceptive action in the writhing test in mice (results not shown), it was submitted for the usual chromatographic procedures (CC, TLC), giving a pure yellow solid (**1**). The spectral data of **1** were very similar to those of GB2a (**2**) (Table I) previously isolated from the roots of *R. gardneriana* (Botta *et al.*, 1984). However, in the <sup>13</sup>C-NMR spectrum, **1**

Table I.  $^1\text{H}$ -NMR (300 MHz) and  $^{13}\text{C}$ -NMR (75 MHz) spectral data of compounds **1** and **2** in  $\text{C}_5\text{D}_5\text{N}$ .

Carbon	Compound <b>1</b>		Compound <b>2</b>	
	$\delta\text{C}$	$\delta\text{H}$	$\delta\text{C}$	$\delta\text{H}$
I-4	197.7	—	197.9	—
II-4	196.0	—	196.4	—
II-7	168.2	—	170.7	—
I-7	167.9	—	168.0	—
II-5	166.3	—	166.4	—
I-5	165.3	—	165.4	—
I-9	164.0	—	164.1	—
II-9	161.7	—	161.9	—
I-4'	159.3	—	159.4	—
II-4'	149.0 <sup>a</sup>	—	147.9 <sup>a</sup>	—
II-3'	148.3 <sup>a</sup>	—	147.6 <sup>a</sup>	—
II-1'	132.6	—	131.0	—
I-2',6'	129.9	7.76 d	130.1	—
I-1'	129.6	—	129.6	—
II-6'	117.7	6.50 m	118.5	6.42 m
II-2'	115.9	7.08 m	116.5	7.12 m
I-3',5'	115.7	7.37 d	115.9	7.34 d
II-5'	112.0	7.08	115.8	7.26
I-10	102.6 <sup>b</sup>	—	102.7 <sup>b</sup>	—
II-10	102.6 <sup>b</sup>	—	102.7 <sup>b</sup>	—
II-8	102.5 <sup>b</sup>	—	102.4 <sup>b</sup>	—
I-8	97.3	6.57	97.3	—
I-6	95.9	6.48	96.0	—
I-2	82.5	6.29 d	82.7	6.26 d
II-2	79.6	5.35 m	79.9	5.50 m
OMe	55.7	3.77 s	—	—
I-3	48.8	5.20 d	48.8	5.25 d
II-3	43.7	2.80 m	43.8	2.82 m
I-5-OH	—	13.5	—	13.2
II-5-OH	—	12.8	—	12.7

<sup>a,b</sup> May be interchanged.(1)  $\text{R}=\text{H}$ ,  $\text{R}'=\text{Me}$ (2)  $\text{R}=\text{R}'=\text{H}$ (3)  $\text{R}''=\text{Me}$ (4)  $\text{R}''=\text{H}$ 

exhibited a signal due to a methoxyl group at  $\delta$  55.7 ppm which was confirmed from  $^1\text{H}$ -NMR spectrum at  $\delta$  3.77 ppm, presumably being attached in ring II. Because of the atropisomerism, a well-known feature of biflavonoids (Häfner and Frahm, 1993; Martinez *et al.*, 1996), the exact localization of the methoxyl group in compound **1** required additional experiments, such as an alkaline hydrolysis. This was carried out under special conditions, according to the methodology previously described (Delle Monache *et al.*, 1967). This provided several compounds including 3-hydroxy-4-methoxy benzoic acid (isovanillic acid), which was detected by spectral data and gas chromatography (co-injection) using an authentic sample thus confirming structure **1**. Another important confirmation of this assumption is a comparison of the  $^{13}\text{C}$ -NMR spectrum with those of two similar flavanones, hesperitin (**3**) and eriodictyol (**4**). The difference in values of C-1' and C-5' (Wagner and Chari, 1976) is very similar to the difference of these carbon atoms in compounds **1** and **2**.

Table II shows that **1** caused graded and significant inhibition of acetic acid-induced abdominal constriction, with an  $\text{ID}_{50}$  value of  $4.5 \mu\text{mol/kg}$  and maximum inhibition of  $80 \pm 4\%$ . It was about 17 to 36 fold-more active than the standard drugs. In the formalin test, **1** inhibited both phases of pain (Table II). However, in contrast to the results previously shown for other biflavonoids present in *R. gardneriana* (Luzzi *et al.*, 1997), compound **1** presented analgesic effects against both the neurogenic (first) and inflammatory (second) phases, but the effect was slightly more pronounced in the late phase of the formalin-induced licking response. The calculated mean  $\text{ID}_{50}$  value for the first phase was  $8.2$  with maximum inhibition of  $62 \pm 2\%$ , whereas the  $\text{ID}_{50}$  value for the second phase was  $6.8 \mu\text{mol/kg}$ , with maximum inhibition of  $86 \pm 3\%$ . It was about 17 fold-more potent than aspirin and paracetamol against the second phase of the formalin test. The activity of **1** in relation to the first phase of the formalin test indicates that the presence of a methoxyl group in the molecule is important to inhibit the chemical mediators involved in the neurogenic pain. This could be related to the increase of hydrophobicity of the molecule.

To determine whether the antinociceptive activity of **1** in formalin-induced pain was related to

Table II. Comparison of the analgesic effect of compound (**1**) with non-steroidal antiinflammatory and analgesic drugs.

Compound	Writhing test	Formalin test	
	ID <sub>50</sub> (μmol/kg.ip)	First phase ID <sub>50</sub> (μmol/kg.ip)	Second phase ID <sub>50</sub> (μmol/kg.ip)
<b>1</b>	4.5 (2.4–8.6) <sup>a</sup> (MI=80 ± 4)	8.2 (4.2–16.1) (MI= 62 ± 2)	6.8 (3.5–12.9) (MI= 86 ± 3)
Aspirin <sup>b</sup>	133 (73–243)	Inactive	123 (77–209)
Dipyrone <sup>b</sup>	162 (88–296)	NT	NT
Indomethacin <sup>b</sup>	76 (41–137)	NT	NT
Paracetamol <sup>c</sup>	125 (104–250)	Inactive	120 (90–161)

<sup>a</sup> Values in parentheses are the 95% confidence limits.<sup>b</sup> From Souza *et al.* (1998).<sup>c</sup> From Cechinel Filho *et al.*, 1998; MI= maximum inhibition.

anti-oedematogenic action, we measured the paw oedema by comparing the difference in weight of the formalin-treated paw and the weight of the control paw (Vaz *et al.*, 1996). The results showed that the analgesic action of **1** against the second phase of the formalin test is not associated with the paw oedema.

Although centrally acting drugs inhibit both phases of the formalin test (Vaz *et al.*, 1996) and

peripheral acting drugs only inhibiting the second phase (Table II), further studies are necessary to elucidate the mechanism of analgesic action of **1**.

#### Acknowledgements

The authors are grateful to Prof. Marcos Sobral for botanical classification of *R. gardneriana*. This work was supported by grants from CNPq/Brazil.

- Bittar M., Souza M. M., Cechinel Filho V., Yunes R. A., Lento R. and Delle Monache F. (2000), Antinociceptive activity of GB-1a, a biflavonoid present in plants of the family Guttiferae. *Planta Med.* **66**, 84–86.
- Botta B., Mac-Quhae M., Delle Monache G., Delle Monache F. and De Mello J. F. (1984), Chemical investigation of the genus *Rheedia*. Biflavonoids and xanthochymol. *J. Nat. Prod.* **47**, 1053.
- Cechinel Filho V., Corrêa R., Vaz Z. R., Calixto J. B., Nunes R. J., Pinheiro T. R., Andricopulo A. D. and Yunes R. A. (1998), Further studies on analgesic activity of cyclic imides. *Il Farmaco*, **53**, 55–57.
- Collier H. D. J., Dinnin L. C., Johnson C. A. and Schneider, C. (1968), The abdominal constriction response and its suppression by analgesic drugs in the mouse. *Br. J. Pharmac. Chemother.* **32**, 295–310.
- Delle Monache F., d'Albuquerque I. L. and Marini Betto G. B. (1967), Su una nuova proanto-cianidina dimera da Ouratea. *Annali di Chimica* **57**, 1364–1371.
- Häfner A. and Frahm A. W. (1993), Biflavonoids from the heartwood of *Garcinia schomburgkiana* and their structural as atropisomers. *Planta Med. (Suppl.)* **59**, A 604.
- Hunskar S., Fasmer O. B. and Hole, K. (1985), Formalin test in mice, a useful technique for evaluating mild analgesics. *J. Neurosci. Methods* **14**, 69–76.
- Luzzi R., Guimarães C. L., Verdi L. G., Simionatto E. L., Delle Monache F., Yunes R. A., Floriani A. E. O. and Cechinel Filho V. (1997), Isolation of biflavonoids with analgesic activity from *Rheedia gardneriana* leaves. *Phytomedicine* **4**, 141–144.
- Martinez E., Moreno-Murillo B. and Delle Monache F. (1996), Fukugetina y fukugiside, biflavonoides de *Clusia guavirensis* Cuatr. *Rev. Col. Quim.* **25**, 15–21.
- Souza M. M., Kern P., Floriani A. E. O. and Cechinel Filho V. (1998), Analgesic properties of a hydroalcoholic extract obtained from *Alternanthera brasiliana*. *Phytother. Res.* **12**, 279–281.
- Vaz Z. R., Cechinel Filho V., Yunes R. A. and Calixto J. B. (1996), Antinociceptive action of 2-(4-bromobenzoyl)-3-methyl-4,6-dimethoxy benzofuran, a novel xanthoxylene derivative on chemical and thermal models of nociception in mice. *J. Pharm. Exp. Ther.* **278**, 304–312.
- Wagner H. and Chari V. M. (1976), <sup>13</sup>C-NMR Spektren natürlich vorkommender Flavonoide. *Tetrahedron Lett.* **21**, 1799–1802.
- Willain Filho A., Breviglieri E., Cechinel Filho V. and Santos A. R. S. (1997), Antinociceptive effect of the hydroalcoholic extract of *Bauhinia splendens* stems. *J. Pharm. Pharmacol.* **49**, 823–827.