Antinociceptive Activity of Niga-ichigoside F_1 from *Rubus imperialis*

R. Niero,[†] V. Cechinel Filho,[†] M. M. Souza,[†] J. L. Montanari,[‡] R. A. Yunes,*[,‡] and F. Delle Monache[§]

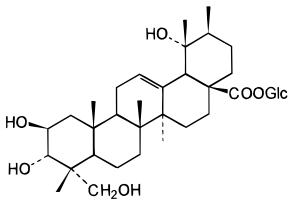
Núcleo de Investigações Químico-Farmacêuticas (NIQFAR), Curso de Farmácia/CCS, Universidade do Vale do Itajaí (UNIVALI), 88.302-202, Itajaí, SC, Brazil, Departamento de Química, Universidade Federal de Santa Catarina (UFSC), 88.040-900, Florianópolis, SC, Brazil, and Centro Chimica Recettori (CNR), Largo F. Vito 1, Rome, Italy

Received September 25, 1998

This work describes the antinociceptive effect of a triterpene glycoside, niga-ichigoside F_1 (**1**), obtained from an EtOAc extract of the aerial parts of *Rubus imperialis*. When evaluated against an HOAc-induced writhing model, it exhibited an ID_{50} value of 3.1 mg/kg (ip). Moreover, in a formalin-induced pain model, both phases of pain were inhibited by compound **1**, with ID_{50} values of 2.6 (first phase) and 2.7 (second phase) mg/kg, (ip), respectively.

The genus *Rubus* consists of many species that are employed in various countries of the world to treat different diseases, especially diabetes.[1,2] Chemical and pharmacological studies have confirmed that some of these plants produce active principles that exert hypoglycemic activity, antibacterial effects against Gram-positive bacteria, and anti-allergic activities against allergic rhinitis, atopic dermatitis, and asthma.[3−5]

Rubus imperialis Chum. Schl. (Rosaceae) grows abundantly in the south of Brazil, being known as "amora-branca", "amora-do-mato", or "amora-brava".[6] It is used in traditional medicine as a remedy to treat diabetes. However, no reports have been found regarding its pharmacological and phytochemical investigation. A MeOH extract of this plant inhibited acetic acid-induced abdominal constrictions in mice, so we have studied its antinociceptive properties in more detail. Thus, different extracts obtained from *R. imperialis* were subjected to pharmacological studies using two classical models of pain in mice, acetic acid-induced writhing and formalin-induced pain. In addition, we have tested a triterpene glycoside F_1, niga-ichigoside (**1**), which was isolated and identified for the first time in this plant, from the EtOAc extract.



1

Table 1 shows the antinociceptive effect of the *R. imperialis* EtOAc extract and compound **1** when given intraperitoneally (ip), on acetic acid-induced abdominal constriction, with aspirin and paracetamol used in comparison. The EtOAc extract caused a dose-dependent antinociceptive effect, with an ID_{50} of 6.8 mg/kg and a maximal inhibition of 94.0%. When given orally (200 mg/kg, ip), it inhibited the abdominal constrictions by 37%. The active principle of the *R. imperialis* EtOAc extract was identified as niga-ichigoside F_1 (**1**) on the basis of the comparison of its spectral data with literature values.[7] This compound significantly inhibited the abdominal constrictions, with an ID_{50} value of 3.1 mg/kg (4.6 μmol/kg). Because of its limited quantity, compound **1** was not analyzed orally. The crude EtOAc extract and niga-ichigoside F_1 (**1**) were more potent than the standards, aspirin or paracetamol (Table l).

When analyzed in a formalin-induced pain model, a useful technique for evaluating neurogenic and inflammatory continuous pain,[8−10] the EtOAc extract and compound **1** displayed significant and dose-related antinociceptive effects against both phases of pain. However, the EtOAc extract was more effective in preventing the inflammatory effects in the second phase than the first phase of formalin-induced nociception. The calculated mean ID_{50} values for EtOAc extract were 35.7 mg/kg, (ip) for the first phase and 23.9 mg/kg (ip) for the second phase, respectively (Table 2). Niga-ichigoside F_1(**1**) exhibited potent antinociceptive effects against both phases of the formalin test, with ID_{50} values (μmol/kg, ip) of 3.9 for the first phase and 4.1 for the second phase. Given that compound **1** is about 1.5% of the weight of the EtOAc extract of *R. imperialis*, other compounds present may also contribute toward the observed antinociceptive activity of the crude extract. Further investigations are necessary to determine the mechanism of the antinociceptive effects of niga-ichigoside F_1 (**1**).

Experimental Section

General Experimental Procedures. The melting point was determined on a Microquímica AP-300 apparatus and is uncorrected, and the optical rotation was run on a Polartronic E polarimeter. The IR spectrum was recorded with a Bomem MB 100 instrument. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian XL-300 NMR spectrometer at 300 and 75 MHz, respectively, in C_5D_5N. The FABMS was obtained on a VG 70−250 S instrument. Si gel 60 (70−230 mesh, Merck) was used for chromatography. TLC was performed on precoated TLC plates with Si gel 60 (F_{254}, Merck).

Plant Material. *Rubus imperialis* was collected at Florianópolis, Brazil, in June 1997, and identified by Dr. Ademir Reis (Department of Botany, UFSC). A voucher specimen was deposited at Barbosa Rodrigues Herbarium under number V. C. Filho 012.

Extraction and Isolation. The dried aerial parts of the plant (1.2 kg) were powdered and macerated with MeOH for 7 days at room temperature. After evaporation of solvent under reduced pressure, the MeOH extract was successively partitioned with hexane, $CHCl_3$, and EtOAc, affording 8.9, 1.2, and 9.4 g of each dried fraction, respectively. Although all extracts

* To whom correspondence should be addressed. Tel.: (048) 331-9219. Fax: (048) 331-9711. E-mail: ryunes@qmc.ufsc.br.
[†] Universidade do Vale do Itajaí.
[‡] Universidade Federal de Santa Catarina.
[§] Centro Chimica Recettori.

10.1021/np9804177 CCC: $18.00
Published on Web 07/02/1999

Table 1. Antinociceptive Effects of the *Rubus imperialis* Ethyl Acetate Extract and Niga-ichigoside F_1 (**1**) against Acetic Acid-induced Abdominal Constrictions in Mice

treatment	ID_{50} (mg/kg, ip)	ID_{50} (μmol/kg, ip)	maximal inhibition (%)[a]
ethyl acetate extract	6.8 (5.3−7.9)		94.0 ± 3.5
niga-ichigoside F_1 (**1**)	3.1 (1.7−5.6)[b]	4.6 (2.6−8.5)	67.6 ± 1.8
aspirin[c]	24.0 (13.0−44.0)	133.0 (73.0−243.0)	83.0 ± 1.4
paracetamol[c]	19.0 (16.0−23.0)	125.0 (104.0−250.0)	88.0 ± 1.0

[a] p values <0.05 were considered as indicative of significance. [b] 95% Confidence limits, with each group representing the mean ± S.E.M. of five to seven experiments. [c] Positive control.

Table 2. Antinociceptive Effects of the *R. imperialis* Ethyl Acetate Extract and Niga-ighigoside F_1 (**1**) in the First (0 to 5 min) and Second (15 to 30 min) Phases in a Formalin Test in Mice

	First phase			Second phase		
treatment	ID_{50} (mg/kg, ip)	ID_{50} (μmol/kg, ip)	maximal inhibition (%)[a]	ID_{50} (mg/kg, ip)	ID_{50} (μmol/kg, ip)	maximal inhibition (%)[a]
ethyl acetate extract	35.7 (18.6−68.5)[b]		72.3 ± 2.0	23.9 (12.5−45.9)		89.9 ± 3.8
niga-ichigoside F_1 (**1**)	2.6 (1.4−4.7)	3.9 (2.1−7.1)	70.4 ± 6.0	2.7 (1.5−4.9)	4.1 (2.3−7.4)	94.0 ± 3.5
aspirin[c]	inactive			22.1 (13.9−37.6)	123.0 (77.0−209.0)	88.0 ± 3.0
paracetamol[c]	inactive			18.1 (13.6−24.3)	120.0 (90.0−161.0)	85.0 ± 3.5

[a] p values < 0.05 were considered as indicative of significance. [b] 95% Confidence limits, with each group representing the mean ± S.E.M. of five to seven experiments. [c] Positive control.

exhibited antinociceptive activity, the EtOAc extract was chosen for further study. On colunm chromatography over Si gel 60, by elution with $CHCl_3$−MeOH (80:20), 150 mg of a major constituent of the EtOAc extract were obtained: white solid pure by TLC; mp 230−231 °C; $[\alpha]^{26}_D$ +11.5°, *c* 1.01, MeOH; FABMS (*m*/*z*) 667 $(M + H)^+$, 689 $(M + Na)^+$, identified as niga-ichigoside F_1 (**1**) on the basis of spectroscopic data (IR, ^1H and ^{13}C NMR, MS) compared to literature values.[7]

Pharmacological Evaluation−Abdominal Constriction Response Caused by Intraperitoneal Injection of Dilute Acetic Acid. The abdominal constriction induced by intraperitoneal injection of HOAc (0.6%) was carried out according to the procedures described previously,[11,12] with minor modifications. Male Swiss mice (25−30 g) were pretreated with EtOAc extract (10−60 mg/kg) and compound **1** (1−10 mg/kg) intraperitoneally or orally (EtOAc extract at 200 mg/kg), 30 min before the HOAc injection. Control animals received a similar volume of 0.9% NaCl (10 mL/kg, ip). All experiments were carried out at 23 ± 2 °C. After challenge, pairs of mice were placed in separate boxes, and the number of constrictions of the abdominal muscles, together with stretching, were counted cumulatively 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 test materials.

Formalin-Induced Pain. The procedure used was essentially similar to that previously described.[9,12] Mice from the same strain as described above were acclimatized to the laboratory for at least 24 h before the experiment and were slightly anesthetized with Et_2O, except when used to analyze the first phase of formalin-induced pain. Next, 20 μL of 2.5% of 0.92% formaldehyde made up with PBS (phosphate buffered solution containing NaCl 137 mM, KCl 2.7 mM, and phosphate buffer 10 mM) were injected subcutaneously under the plantar surface of the left hindpaw with a Hamilton syringe. Animals were treated 60 min before the formalin injection with normal saline (10 mL/kg, ip), with EtOAc extract (10−60 mg/kg, ip) or with compound **1** (1−10 mg/kg, ip) obtained from *R. imperialis*. After intraplantar irritant application, the animals were then placed immediately 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. Two mice (control and treated) were simultaneously observed from 0 up to 30 min after formalin injection. The initial nociceptive scores normally peaked 5 min (first phase, representing the neurogenic pain) and 15−30 min (second phase, representing the inflammatory pain) after formalin injection.[8]

Statistical Analysis. The results are presented as mean ± S. E. M., except the mean ID_{50} values (i.e., the dose of the test material reducing the antinociceptive response by 50% relative to the control value), which are reported as geometric means accompanied by their respective 95% confidence limits. The statistical significance among groups was first analyzed by ANOVA, then followed by up Dunnett's multiple comparison test to check the significance between groups; p values less than 0.05 were considered significant. The ID_{50} values were determined by graphical interpolation from individual experiments.

Acknowledgment. The authors are grateful to Prof. Dr. A. Reis for the botanical identification and to CNPq, CAPES, and UNIVALI/Brazil for financial support.

References and Notes

(1) Alonso, R.; Cadavid, I.; Calleja, J. M. *Planta Med.* **1980**, *46 (Suppl.)*, 102−106.
(2) Zonta, C. M.; Ruediger, R. *Monografia de Estágio, Curso de Farmácia*; Universidade do Vale do Itajaí (UNIVALI): Itajaí, SC, Brazil, 1996.
(3) Swanston-Flatt, S. K.; Day, C.; Bailey, C. J.; Flatt, P. R. *Diabetologia* **1990**, *33* 462−464.
(4) Richards, R. M.; Durhan, E. D. G.; Liu, X. *Planta Med.* **1994**, *60*, 471−473.
(5) Nakahara, K. EP 0727218, Sep. 30, 1996, YY pp.; *Chem. Abstr.* **1996**, *125*, 230780h
(6) Cirilo, V. K. *Manual de Plantas Medicinais*, 44th ed.; Assessoar, Francisco Beltrão: Paraná, Brazil, 1993.
(7) Seto, T.; Tanaka, T.; Tanaka, O.; Naruhashi, N. *Phytochemistry* **1984**, *23*, 2829−2834.
(8) Hunskaar, S.; Hole, K. *Pain* **1987**, *30*, 103−114.
(9) Hunskaar, S.; Fasmar, O. B.; Hole, K. *J. Neurosci. Methods* **1985**, *14*, 69−76.
(10) Vaz, Z. R.; Cechinel Filho, V.; Yunes, R. A.; Calixto J. B. *J. Pharmacol. Exp. Ther.* **1996**, *278*, 304−312.
(11) Collier, H. D. J.; Dinnen, L. C.; Johnson, C. A.; Schneider, C. *Br. J. Pharmacol.* **1968**, *32*, 295−310.
(12) Santos, A. R. S.; Cechinel Filho, V.; Niero, R.; Pizzolatti, M. G.; Delle Monache, F. Yunes, R. A.; Calixto, J. B. *Planta Med.* **1995**, *61*, 329−332.

NP9804177