JPP Journal of Pharmacy and Pharmacology

JPP 2006, 58: 107–112

Received March 3, 2005
Accepted September 5, 2005
DOI 10.1211/jpp.58.1.0013
ISSN 0022-3573

Antinociceptive properties of coumarins, steroid and dihydrostyryl-2-pyrones from *Polygala sabulosa* (Polygalaceae) in mice

Flavia Carla Meotti, Juliana V. Ardenghi, Juliana B. Pretto, Márcia M. Souza, Janaína d' Ávila Moura, Anildo Cunha Junior, Cristian Soldi, Moacir Geraldo Pizzolatti and Adair R. S. Santos

Departamento de Química, Universidade Federal de Santa Maria, 97110-000, Santa Maria, RS - Brazil

Flavia Carla Meotti

Curso de Farmácia, Centro de Ciências da Saúde, Universidade do Vale do Itajaí, Itajaí 88302-202, SC - Brazil

Juliana V. Ardenghi, Juliana B. Pretto, Márcia M. Souza

Departamento de Química, Universidade Federal de Santa Catarina, Florianópolis 88040-900, SC - Brazil

Anildo Cunha Junior, Cristian Soldi, Moacir Geraldo Pizzolatti

Departamento de Ciências Fisiológicas, Universidade Federal de Santa Catarina, Florianópolis 88040-900, SC - Brazil

Flavia Carla Meotti, Janaína d' Ávila Moura, Adair R. S. Santos

Correspondence: A. R. S. Santos, Departamento de Ciências Fisiológicas, Universidade Federal de Santa Catarina, Campus Universitário – Trindade, 88040-900, Florianópolis, SC, Brazil. E-mail: arssantos@ccb.ufsc.br

Funding and acknowledgments: The authors are grateful to Prof. Dr Olavo de Araújo Guimarães for botanical classification of *Polygala sabulosa*. This study was supported by grants from CNPq, PRONEX, CAPES and FAPESC, Brazil. A. C. Junior and C. Soldi are undergraduate chemistry students and F. C. Meotti is a PhD student in toxicological biochemistry. They thank CNPq and CAPES for fellowship support.

Abstract

We have investigated the possible antinociceptive action of the extract, fractions and pure compounds obtained from the whole plant *Polygala sabulosa* A. W. Bennett (Polygalaceae) in acetic acid-induced visceral pain in mice. Intraperitoneal injection of animals with the hydroalcoholic extract and fractions (CH_2Cl_2, EtOAc, *n*-BuOH, aqueous fraction) (1–100 mg kg^{-1}) caused a dose-related and significant inhibition of the acetic acid-induced visceral nociceptive response. The CH_2Cl_2, EtOAc and *n*-BuOH fractions were more potent than the hydroalcoholic extract and aqueous fraction. The isolated compounds dihydrostyryl-2-pyrones (**1**, **2, 3**), styryl-2-pyrone (**7**), α-spinasterol (**9**), scopoletin (**10**) and two esters of the coumarin (scopoletin) obtained semisynthetically, acetylscopoletin (**10a**) and benzoylscopoletin (**10b**) (0.001–10 mg kg^{-1}), exhibited significant and dose-related antinociceptive effects against acetic acid-induced visceral pain. The results distinguished, for the first time, the extract, fractions and pure compounds obtained from *P. sabulosa* that produced marked antinociception against the acetic acid-induced visceral nociceptive response, supporting the ethnomedical use of *P. sabulosa*.

Introduction

Polygala sabulosa A. W. Bennett (Polygalaceae), popularly known as 'timutu-pinheirinho', is a small herb growing in the Southern Meridional Highlands of Brazil (Wurdack & Smith 1971). The plants of the genus *Polygala* are used in folk medicine for the treatment of several pathologies, including disorders of the bowel and kidney, as a tonic remedy, and as a topical anaesthetic and expectorant (Wasicky 1945). Apart from these medicinal uses, there are reports showing antiviral (Cyong et al 2000), trypanocidal (Pizzolatti et al 2003), tumour inhibitor (Mak et al 2001), hypoglycaemic (Kako et al 1996) and neuroprotective (Kwon et al 2004) effects of some *Polygala* species.

We have reported previously that the hydroalcoholic extract and xanthones obtained from *P. cyparissias* elicited pronounced antinociceptive effects in chemical and mechanical nociception models (De Campos et al 1997). In addition, several species of plants belonging to the genus *Polygala*, including *P. cyparissias*, *P. linoides*, *P. campestris*, *P. paniculata* and *P. aspalata*, are extensively distributed in Santa Catarina. These plants contain high concentrations of methyl salicylate, which is responsible for a characteristic odour of their roots (Wasicky 1945). Chemical studies carried out on some of the species belonging to the genus *Polygala* have demonstrated the presence of many classes of constituents, such as coumarins, saponins, lignans, flavonoids and mainly xanthones (Pinheiro et al 1998; Zhang et al 1998; Cristiano et al 2003). More recently, Pizzolatti et al (2000, 2004) have reported the isolation of 7-prenyloxy-6-methoxycoumarin, photohypericin, four styryl-2-pyrones and three dihydrostyryl-2-pyrones from *P. sabulosa*. However, to date no pharmacological study has been carried out on this species. Furthermore, whole plant from *P. sabulosa* is used in folk medicine; hence, this study has examined the possible antinociceptive action of the hydroalcoholic extract, fractions and isolated compounds in the acetic acid-induced visceral nociceptive response, a classical chemical model of nociception, in mice.

Materials and Methods

Plant material

Polygala sabulosa A. W. Bennett (Polygalaceae) was collected in Rancho Queimado (Santa Catarina, Brazil) in November 1997. It was identified by Prof. Dr. Olavo de Araújo Guimarães. A voucher specimen was deposited at the Herbarium of the Botany Department, Universidade Federal do Paraná, PR, under the number 19640.

Extraction and isolation

Whole plants (500 g) were air-dried, ground to powder and extracted with 96% ethanol at room temperature for 14 days. The alcoholic extract was then dried by evaporation under reduced pressure and the crude extract (135 g) obtained was partitioned into hexane-, CH_2Cl_2-, EtOAc- and *n*-BuOH-soluble fractions. This gave five distinct hexane-soluble (16.1 g), CH_2Cl_2-soluble (28 g), AcOEt-soluble (16.8 g), *n*-BuOH-soluble (17.6 g) and aqueous (47 g) fractions. These fractions were screened for antinociceptive activity and the CH_2Cl_2 and EtOAc active fractions were subjected to fractionation by column chromatography. The CH_2Cl_2-soluble fraction (16 g) was subjected to column chromatography over silica gel (4 × 30 cm). A total of 56 fractions (100 mL each) were eluted with mixtures of the hexane–EtOAc from 100:0 to 0:100 and then EtOAc–EtOH 1:1. The elutes were monitored by TLC (viewed by spraying with 1% vanillin/5% H_2SO_4/EtOH reagent followed by heating at 110°C) and similar fractions were combined. Combined fractions 9–14 were recrystallized from acetone to give α-spinasterol; **9** (25 mg; optical rotation of $[\alpha]^{20}_{D} - 2.5°$). Combined fractions 21–26 were dissolved in EtOAc (50 mL) and treated under heating and shaking with activated charcoal (1 g). After filtration, drying (anhydrous Na_2SO_4) and removal of the solvent, the white residue was purified by recrystallization from hexane-EtOAc (3:1) to obtain compound **8** (7-prenyloxy-6-methoxycoumarin) (530 mg). Fraction 29 was purified by recrystallization from acetone to yield compound **1** (17 mg). The combined fractions 30–36 gave yellow powders containing the compounds **1**, **2**, **3** and **4** (by GC-MS) that had been described previously for this species (Pizzolatti et al 2000). Further flash chromatography over silica gel 230–400 mesh (2 × 25 cm) eluted with hexane–EtOAc (3:1, 190 mL) afforded compounds **2** (150 mg) and **3** (25 mg). The combined fractions 38–42 (1.8 g) were resubmitted to chromatography over silica gel (2.5 × 25 cm) using hexane–EtOAc 3:2 to give 575 mg of a grey powder. Further chromatography of the latter over silica gel (2 × 15 cm; hexane–EtOAc 3:2), following purification by recrystallization from acetone, yielded 560 mg scopoletin (**10**). The EtOAc-soluble fraction (10 g) was further subjected to column chromatography over silica gel (3 × 30 cm) using hexane–EtOAc (100:0 to 0:100) and EtOAc–EtOH (100:0 to 0:100) to give 72 fractions of 50 mL each. The GC-MS analysis of the combined fractions 8–13 produced five peaks for compounds **2**, **4**, **5**, **6** and **7** as described previously (Pizzolatti et al 2000, 2004). Styryl-2-pyrone (**7**) was the major component among fractions 8–13 and this was further purified by flash chromatography (2 × 2.5 cm, silica gel 230–400 mesh) eluted with hexane–EtOAc (3:1), to give 185 mg compound **7**.

Chemical derivatives

Acetylation of scopoletin

Acetic anhydride (1.5 mL) and a catalytic amount of 4-dimethylaminopyridine (DMAP) were added to a solution of 40.0 mg scopoletin in ethyl acetate (5.0 mL). The reaction mixture was stirred for 40 min at room temperature. The solution was evaporated with additions of ethanol, and the crude product was filtered over silica gel and evaporated to dryness yielding acetylscopoletin.

Benzoylation of scopoletin

Benzoyl chloride (0.05 mL) was added to a solution of 60.0 mg scopoletin in pyridine (1.5 mL). The reaction mixture was stirred for 5 min at room temperature. Ethanol was added and the solution was evaporated. Column chromatography on silica gel gave benzoylscopoletin.

Abdominal constriction caused by intraperitoneal injection of 0.6% acetic acid

Male Swiss mice (25–35 g) were kept in an automatically-controlled temperature room (23 ± 2°C) in 12-h light–dark cycles, with water and food freely available. Animals were acclimatized to the laboratory for at least 2 h before testing and were used once for experiments. All experiments were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals as previous specified (Zimmermann 1983). The experiments were approved by the local Ethics Committee of this Institution (23080.011700/2005–03/UFSC). The numbers of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate consistent effects of the drug treatments.

The procedure used for acetic acid (0.6%)-induced abdominal constriction was essentially similar to that described previously (Collier et al 1968; Santos et al 1995). Animals were pretreated intraperitoneally (i.p) with extract or fraction (1–100 mg kg^{-1}): dihydrostyryl-2-pyrone (**1**, **2**, **3**); styryl-2-pyrone (**7**) or 7-prenylxy-6-methoxycoumarin (**8**) (1–10 mg kg^{-1}); α-spinasterol (**9**), α-scopoletin (**10**), acetylscopoletin (**10a**) or benzoylscopoletin (**10b**) (0.001–10 mg kg^{-1}) 30 min before testing. Due to the limited amount of these compounds, it was not possible to test them orally. Control animals received the same volume of vehicle (10 mL kg^{-1}, i.p). After challenge, mice were placed in separate boxes and the number of abdominal constrictions was cumulatively counted over 20 min.

Drugs

The drugs used were acetic acid and Tween 80 (Merck AG, Darmstadt, Germany). All other reagents used were of a high grade of purity. The degree of purity of the compounds obtained from *P. sabulosa* was >98%. The hydroalcoholic extract, fractions and pure compounds from *P. sabulosa* were dissolved in Tween 80 and diluted just before use in 0.9%

NaCl. The final concentration of Tween 80 did not exceed 5% and did not have any effect itself.

Statistical analysis

The results were presented as means ± s.e.m. of six to eight animals. The significance of difference between groups was analysed by means of analysis of variance followed by Newman–Keuls' multiple comparison test; $P < 0.05$ was considered as indicative of significance. The ID50 values (i.e. the dose of the extract, fractions or compounds necessary to reduce the pain response by 50% in relation to the control value) were reported as geometric means accompanied by their respective 95% confidence limits. The ID50 values were determined by linear regression from individual experiments with linear regression GraphPad Software.

Results

P. sabulosa, whole plant, was extracted with 96% EtOH at room temperature and the hydroalcoholic extract obtained was partitioned into hexane-, CH_2Cl_2-, EtOAc-, *n*-butanol- and aqueous fractions. Repeated fractionation over silica gel columns of the CH_2Cl_2- and EtOAc-fractions yielded compounds **1**–**8** that had been previously described for this species (Pizzolatti et al 2000, 2004), α-spinasterol (**9**) and scopoletin (**10**) (Figure 1). The structures of **1**–**10** were elucidated by comparison of their spectroscopic data (^1H, ^{13}C NMR) with data in the literature. The α-spinasterol and scopoletin from *P. sabulosa* were reported here for the first time.

Intraperitoneal injection of animals with hydroalcoholic extract and fractions (CH_2Cl_2, EtOAc, *n*-BuOH, aqueous fractions) did not produce any irritation by themselves (result not shown), but did cause a dose-related and significant inhibition of the acetic acid-induced visceral nociceptive response in mice.

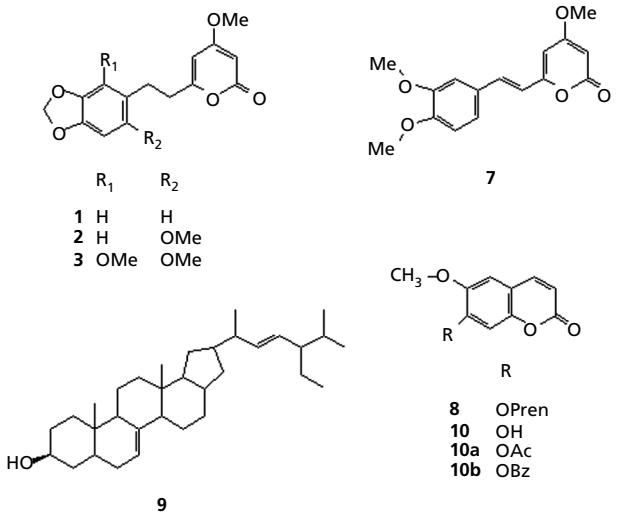

Figure 1 Chemical structure of dihydrostyryl-2-pyrones (**1**, **2**, **3**); styryl-2-pyrone (**7**); 7-prenyloxy-6-methoxycoumarin (**8**); α-spinasterol (**9**); scopoletin (**10**) and its structural derivatives acetylscopoletin (**10a**) and benzoylscopoletin (**10b**).

The calculated mean ID50 value (95% confidence limits) and inhibition values are shown in Table 1. At the ID50 level, CH_2Cl_2, EtOAc and *n*-BuOH soluble fractions were approximately 1.7- to 3.7-fold more potent than hydroalcoholic extract, aqueous soluble fractions and two well-known anti-inflammatory and analgesic drugs (paracetamol and aspirin) (Table 1). Considering that CH_2Cl_2 and EtOAc were the most potent fractions, these were selected for the isolation of active constituents.

In the next stage of this study, the effect of isolated compounds was examined in the acetic acid-induced abdominal constriction test. The dihydrostyryl-2-pyrones (**1**, **2**, **3**) and styryl-2-pyrone (**7**) exhibited significant and dose-related antinociceptive actions in this model of visceral pain. The calculated mean ID50 value (and 95% confidence limits) and inhibition values are shown in Table 2. At the ID50 level, **1**, **2**, and **3** were approximately 1.3- to 2.2-fold more potent than the CH_2Cl_2 fraction (original fraction). Compound **7** was two times more potent than EtOAc (original fraction). The **1**, **2**, **3** and **7** compounds were 3.4- to 7.3-fold more potent than two well-known anti-inflammatory and analgesic drugs (paracetamol and aspirin) (Tables 1 and 2). However, compound **8** was less potent in inhibiting the acetic acid-induced nociception, returning a value of 46 ± 10% at 10 mg kg^{-1}.

Table 1 Inhibition by extract and fractions from *Polygala sabulosa* on acetic acid-induced abdominal constriction

Drugs	ID50 (mg kg^{-1})	Inhibition (%)
Hydroalcoholic extract	21.3 (16.4–27.5)[a]	93 ± 5
CH_2Cl_2 soluble fraction	7.6 (5.2–11.2)	78 ± 7
EtOAc soluble fraction	7.2 (6.2–8.4)	93 ± 4
n-BuOH soluble fraction	11.0 (8.1–15.0)	84 ± 7
Aqueous soluble fraction	27.0 (16.4–44.4)	92 ± 4
Paracetamol[b]	18.8 (15.7–22.6)	88 ± 1
Aspirin[b]	24.0 (13.1–43.8)	83 ± 2

Mice (n = 6–8 per group) were treated with extract or fractions 30 min (i.p.) before acetic acid administration. [a]95% confidence limits. [b]Data from Vaz et al (1996).

Table 2 Inhibition by dihydrostyryl-2-pyrones (**1**, **2**, **3**), styryl-2-pyrone (**7**), 7-prenyloxy-6-methoxycoumarin (**8**) α-spinasterol, scopoletin and its structural derivatives on acetic acid-induced abdominal constriction

Drugs	ID50 (mg kg^{-1})	Inhibition (%)
1	4.9 (3.8–6.2)[a]	85 ± 7
2	3.5 (3.0–4.1)	81 ± 9
3	5.5 (4.2–7.2)	85 ± 5
7	3.3 (2.9–3.8)	97 ± 2
8	ND	46 ± 10
α-Spinasterol	0.07 (0.05–0.09)	99 ± 1
Scopoletin	0.06 (0.04–0.08)	96 ± 2
Acetylscopoletin	1.56 (1.05–2.32)	83 ± 5
Benzoylscopoletin	0.032 (0.014–0.073)	81 ± 8
Paracetamol[b]	18.8 (15.7–22.6)	88 ± 1
Aspirin[b]	24.0 (13.1–43.8)	83 ± 2

Mice (n = 6–8 per group) were treated with compounds 30 min (i.p.) before acetic acid administration. [a]95% confidence limits. [b]Data from Vaz et al (1996). ND, not determined.

The results in Figure 2A and B showed that α-spinasterol and scopoletin caused a dose-related inhibition of the acetic acid-induced visceral nociceptive response. The ID50 and inhibition values are shown in Table 2. At the ID50 level, α-spinasterol and scopoletin were approximately 55- to 91-fold more potent than all the other compounds isolated from *P. sabulosa* (Table 2). Furthermore, α-spinasterol and scopoletin were approximately 313- to 400-fold more potent than paracetamol and aspirin (Table 2). Benzoylscopoletin and acetylscopoletin, which were structurally derived from scopoletin, were examined in the acetic acid-induced abdominal constriction test. Both derivatives caused significant and dose-related inhibition of the acetic-acid induced visceral nociceptive response (Figure 2C and D). At the ID50 level, benzoylscopoletin was two times more potent than scopoletin. Acetylscopoletin, on the other hand, was 26-fold less potent than scopoletin (Table 2).

Discussion

The results showed for the first time that hydroalcoholic extract, fractions and active compounds from *P. sabulosa*, administered intraperitoneally to mice produced significant antinociceptive action when assessed in acetic acid-induced visceral pain.

The acetic acid-induced visceral nociceptive response in mice is regarded as a typical model of inflammatory pain and has long been used as a screening tool for evaluation of analgesic or anti-inflammatory agents (Collier et al 1968; Vineger et al 1979). It has been suggested that acetic acid acts by releasing endogenous inflammatory mediators (i.e. kinins, substance P and prostanoids) that stimulate the primary sensory neurons (Collier et al 1968; Vineger et al 1979; Ikeda et al 2001). It is sensitive to non-steroidal anti-inflammatory drugs (NSAIDs) and to narcotics and other centrally acting drugs (Collier et al 1968; Vineger et al 1979; Vaz et al 1996; Santos et al 1998; Reichert et al 2001).

Ribeiro et al (2000) demonstrated that the nociceptive activity of acetic acid may be due to cytokine release, such as TNF-α, interleukin-1β and interleukin-8, by resident peritoneal macrophages and mast cells. Feng et al (2003) demonstrated that intraperitoneal injection of acetic acid induced an increase in the concentration of glutamate and aspartate in the cerebrospinal fluid.

We have reported that CH_2Cl_2, EtOAc and *n*-butanol fractions obtained from *P. sabulosa* inhibited, in a dose-dependent manner, the nociception induced by acetic acid. When compared with well-known NSAIDs, aspirin and paracetamol, the CH_2Cl_2, EtOAc and *n*-butanol fractions were 1.7 to 3.7-fold more potent to inhibit the acetic acid-induced visceral nociceptive response, i.e. the same inhibition demonstrated by aspirin and paracetamol was observed by CH_2Cl_2, EtOAc and *n*-butanol fractions at 1.7- to 3.3-times lower doses. This potency was estimated by the ID50 value (Table 1). Furthermore, all the isolated compounds, with the exception of compound **8**, displayed similar analgesic actions on acetic acid-induced pain. Thus, dihydrostyryl-2-pyrones (**1**, **2**, **3**) isolated from the CH_2Cl_2 fraction and styryl-2-pyrone (**7**) isolated from the EtOAc fraction were respectively 1.3- to

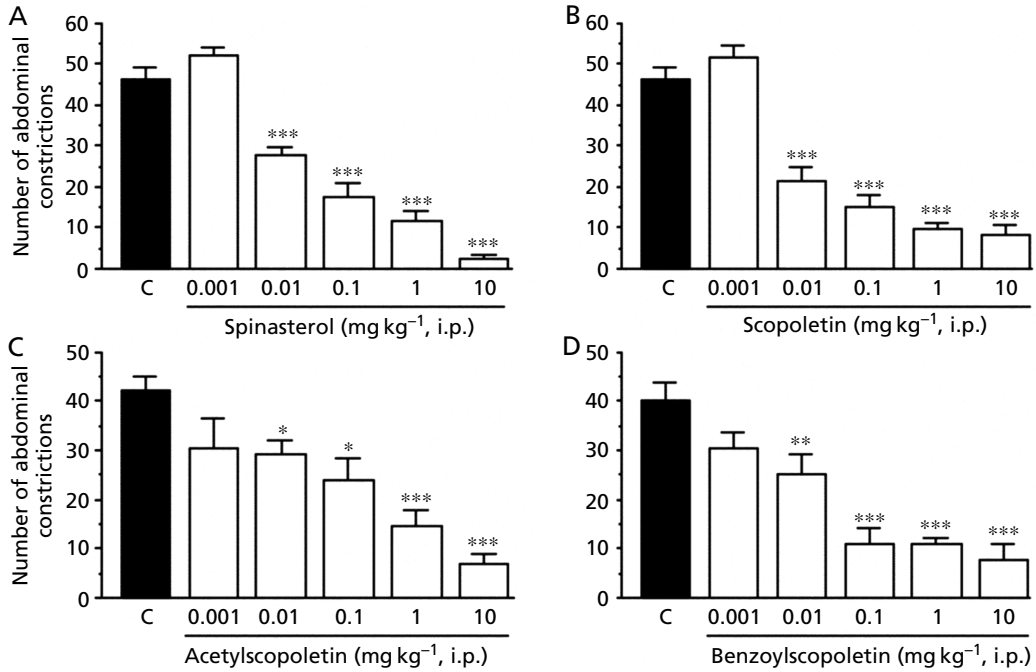

Figure 2 Effect of α-spinasterol **(A)**, scopoletin **(B)**, acetylscopoletin **(C)** and benzoylscopoletin **(D)** against the acetic acid-induced visceral nociceptive response. The animals were treated with compounds at different doses or vehicle (10 mL kg^{-1}), intraperitoneally, 30 min before acetic acid injection. Each column represents the mean ± s.e.m. of six to eight animals. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with control groups (C).

2.2-fold and 2.3-fold more potent than CH_2Cl_2 and EtOAc. As a result, it could be suggested that these compounds contributed, at least in part, to the antinociceptive effect of CH_2Cl_2 and EtOAc fractions. The main finding of this study was the demonstration that two compounds, α-spinasterol (**9**) and scopoletin (**10**), present in the CH_2Cl_2 fraction, were the most potent and efficacious in inhibiting the acetic acid-induced visceral nociceptive response. At the ID50 level, compounds **9** and **10** were approximately 55- to 91-fold more potent than all other compounds isolated from *P. sabulosa*, and 313- to 400-fold more potent than paracetamol and aspirin.

There is evidence showing that α-spinasterol exhibits antigenotoxic and anticarcinogenicity activity (Villasenor et al 1996; Villasenor & Domingo 2000). Furthermore, α-spinasterol exerts a concentration-dependent inhibition of rat ileum spontaneous contraction (Mata et al 1997). Smooth muscle contraction is regulated, primarily, by intracellular Ca^{2+} signal. Additionally, it is well established that an increase in cytosolic Ca^{2+} represents a key step for neurotransmitter release, modulation of cellular membrane excitability, as well as activation of intracellular proteins (Somlyo & Somlyo 1994; Ward 2004). Evidence has accumulated for the involvement of calcium ions and calcium channels in nociception (Prado 2001; Cervero & Laird 2003). In addition, there are data indicating that calcium channels expressed in visceral primary afferent nociceptive neurons may contribute to the sensitization observed in visceral pain states (Cervero & Laird 2003). Thus, those previous findings and these results might indicate that the antinociceptive action of α-spinasterol in the acetic acid abdominal constriction test could be due to inhibition of calcium influx or of blocked calcium channels. However, additional studies are necessary to address this hypothesis.

Our results showed that intraperitoneal administration of scopoletin produced a strong and dose-dependent inhibition of the nociceptive response to acetic acid. Recent papers reported that scopoletin comprised antioxidant, anti-inflammatory and antinociceptive properties (Tanaka et al 1977; Muschietti et al 2001; Ng et al 2003). Scopoletin caused a reduction of eicosanoid release from mouse peritoneal macrophages, which was stimulated by calcium-ionophore A23187 (Silván et al 1996). More recently, Kim et al (2004) demonstrated that scopoletin significantly reduced prostaglandin E_2, tumour necrosis factor-α, interleukin-1β (IL-1β), IL-6 production and suppressed cyclooxygenase-2 (COX-2), but not COX-1 expression, in RAW 264.7, induced by lipopolysaccharide. Such findings suggested that scopoletin could produce antinociceptive action through inhibition of COX-2 and consequently decrease prostaglandin synthesis, or by inhibition of cytokine release from resident peritoneal cells. This possibility requires further study.

Of particular importance, scopoletin (**9**) produced powerful inhibition of acetic acid-induced visceral pain; however, 7-prenyloxy-6-methoxycoumarin (**8**) did not display this effect. There was a structural similarity between scopoletin and **8**, except that the latter contained a prenyl group binding to ring coumarin. The discrepancy between the effects of scopoletin and 7-prenyloxy-6-methoxycoumarin (**8**) could be explained, in part, by the presence of the prenyl group, which can alter pharmacokinetic or pharmacodynamic properties of a compound.

Two structural derivatives of scopoletin, acetylscopoletin (**10a**) and benzoylscopoletin (**10b**) exhibited antinociceptive activity also. Benzoylscopoletin (**10b**) decreased the acetic acid-induced abdominal constriction 2-fold more potently than the original molecule. Hence, the addition of a benzoyl group increased antinociceptive properties of the compound. It may be that the benzoyl group increased the absorption of the compound or facilitated its binding to the active site. On the other hand, the addition of an acetyl group, as in acetylscopoletin, decreased the antinociceptive properties of the compound by approximately 26-fold compared with the original coumarin. Although there is some similarity between benzoylscopoletin and acetylscopoletin, minor changes in the molecule were associated with altered antinociceptive properties, possibly as a result of altered bioavailability of the compounds. Thus, further investigations are needed to investigate the relationship between structure and activity of the compounds.

Conclusion

The results demonstrated for the first time that extract, fractions and pure compounds obtained from *P. sabulosa* produced dose-related antinociception in the acetic acid-induced model of visceral pain in mice, giving support to the ethnomedical use of *P. sabulosa*. Such findings are of interest because they support, at least partly, the notion that scopoletin and α-spinasterol are the active principles present in *P. sabulosa*. Thus, scopoletin and α-spinasterol or its derivative might be useful in the development of new analgesic drugs for the management of visceral and inflammatory pain.

References

Cervero, F., Laird, J. M. A. (2003) Role of ions channels in mechanisms controlling gastrointestinal pain pathways. *Curr. Opin. Pharmacol*. **3**: 608–612

Collier, H. O. J., Dinneen, J. C., Johnson, C. A., Schneider, C. (1968) The abdominal constriction response and its suppression by analgesic drugs in the mouse. *Br. J. Pharmacol. Chemother*. **32**: 295–310

Cristiano, R., Pizzolatti, M. G., Delle Monache, F., Rezende, C. M., Branco, A. (2003) Two xanthones from *Polygala paniculata* and confirmation of the 1-hydroxy-2,3,5-trimethoxy-xanthone at trace level by HRGC-MS. *Z. Naturforsch*. **58**: 490–494

Cyong, J. C., Ki, S. M., Iijima, K., Kobayashi, T., Furuya, M. (2000) Clinical and pharmacological studies on liver diseases treated with Kampo herbal medicine. *Am. J. Chin. Med*. **28**: 351–360

De Campos, R. O. P., Santos, A. R. S., Vaz, Z. R., Pinheiro, T. R., Pizzolatti, M. G., Cechinel, V., Delle Monache, F., Yunes, R. A., Calixto, J. B. (1997) Antinociceptive properties of the hydroalcoholic extract and preliminary study of a xanthone isolated from *Polygala cyparissias* (Polygalaceae). *Life Sci*. **61**: 1619–1624

Feng, Y., Cui, M., Willis, W. (2003) Gabapentin markedly reduces acetic acid-induced visceral nociception. *Anesthesiology* **98**: 729–733

Ikeda, Y., Ueno, A., Naraba, H., Oh-ishi, S. (2001) Involvement of vanilloid receptor VR1 and prostanoids in the acid-induced writhing responses of mice. *Life Sci*. **69**: 2911–2919

Kako, M., Miura, T., Nishiyama, Y., Ichimaru, M., Moriyasu, M., Kato, A. (1996) Hypoglycemic effect of the rhizomes of *Polygala senega* in normal and diabetic mice and its main component, the triterpenoid glycoside senegin-II. *Planta Med*. **62**: 440–443

Kim, H., Jang, S. I., Kim. Y., Chung, H., Yun, Y., Kang, T., Jeong, O., Kim, Y. (2004) Scopoletin suppresses pro-inflammatory cytokines and PGE2 from LPS-stimulated cell line, RAW 246.7 cells. *Fitoterapia* **75**: 261–266

Kwon, Y. S., Nabeshima, T., Shin, E. J., Chun, W., Jhoo, J. H., Jhoo, W. K., Wie, M. B., Jang, C. G., Chung, H., Sung, Y. E., Kim, H. C. (2004) PAP 9704, a Korean herbal medicine attenuates methamphetamine-induced hyperlocomotion via adenosine A_{2A} receptor stimulation in mice. *Biol. Pharm. Bull*. **27**: 906–909

Mak, N. K., Lung, H. L., Wong, R. N., Leung, H. W., Tsang, H. Y., Leung, K. N. (2001) Expression of protein kinase C isoforms in euxanthone-induced differentiation of neuroblastoma cells. *Planta Med*. **67**: 400–405

Mata, R., Rojas, A., Acevedo, L., Estrada, S., Calzada, F., Rojas, I., Bye, R., Linares, E. (1997) Smooth muscle relaxing flavonoids and terpenoids from Conyza filaginoides. *Planta Med*. **63**: 31–35

Muschietti, L., Gorzalczany, S., Ferraro, G., Acevedo, C., Martino, V. (2001) Phenolic compounds with anti-inflammatory activity from *Eupatorium buniifolium. Planta Med*. **67**: 743–744

Ng, T. B., Liu, F., Lu, Y., Cheng, C. H., Wang, Z. (2003) Antioxidant activity of compounds from the medicinal herb *Aster tataricus*. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol*. **136**: 109–115

Pinheiro, T. R., Cechinel, F. V., Santos, A. R. S., Calixto, J. B., Delle Monache, F., Pizzolatti, M. G., Yunes, R. A. (1998) Three xanthones from *Polygala cyparissias*. *Phytochemistry* **48**: 725–728

Pizzolatti, M. G., Luciano, C., Delle Monache, F. (2000) Styryl- and dihydrostyryl-2-pyrones derivatives from *Polygala sabulosa*. *Phytochemistry* **55**: 819–822

Pizzolatti, M. G., Koga, A. H., Grisard, E. C., Steindel, M. (2003) Trypanocidal activity of extracts from Brazilian Atlantic Rain Forest plant species. *Phytomedicine* **10**: 422–426

Pizzolatti, M. G., Cunha, Jr. A, Pereira, W. S., Delle Monache, F. (2004) A new styryl-2-pyrone derivative from *Polygala sabulosa*. *Biochem. Syst. Ecol*. **32**: 603–606

Prado, W. A. (2001) Involvement of calcium in pain and antinociception. *Braz. J. Med. Biol. Res*. **34**: 449–461

Reichert, J. A., Daughters, R. S., Rivard, R., Simone, D. A. (2001) Peripheral and preemptive opioid antinociception in a mouse visceral pain model. *Pain* **89**: 221–227

Ribeiro, R. A., Vale, M. L., Thomazzi, S. M., Paschoalato, A. B. P., Poole, S., Ferreira, S. H., Cunha, F. Q. (2000) Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. *Eur. J. Pharmacol*. **387**: 111–118.

Santos, A. R. S., Niero, R., Filho, V. C., Yunes, R. A., Pizzolatti, M. G., Delle Monache, F., Calixto, J. B. (1995) Antinociceptive properties of steroids isolated from *Phyllanthus corcovadensis* in mice. *Planta Med*. **61**: 329–332

Santos, A. R. S., Vedana, E. M., De Freitas, G. A. (1998) Antinociceptive effect of meloxicam, in neurogenic and inflammatory nociceptive models in mice. *Inflamm. Res*. **47**: 302–307

Silván, A. M., Abad, M. J., Bermejo, P., Sollhuber, M., Villar, A. (1996) Antiinflammatory activity of coumarins from *Santolina oblongifolia*. *J. Nat. Prod*. **59**: 1183–1185

Somlyo, A. P., Somlyo, A. V. (1994) Signal transduction and regulation in smooth muscle. *Nature* **372**: 231–236

Tanaka, S., Ikeshiro, Y., Tabata, M., Konoshima, M. (1977) Antinociceptive substances from the roots of *Angelica acutiloba. Arzneimittelforschung* **27**: 2039–2045

Vaz, Z. R., Cechinel, V., Yunes, R. A., Calixto, J. B. (1996) Antinociceptive action of 2-(4-bromobenzoyl)-3-methyl-4,6-dimethoxy benzofuran, a novel xanthoxyline derivative on chemical and thermal models of nociception in mice. *J. Phamacol. Exp. Ther*. **278**: 304–312

Villasenor, I. M., Domingo, A. P. (2000) Anticarcinogenicity potential of spinasterol isolated from squash flowers. *Teratog. Carcinog. Mutagen*. **20**: 99–105

Villasenor, I. M., Lemon, P., Palileo, A., Bremner, J. B. (1996) Antigenotoxic spinasterol from *Cucurbita maxima* flowers. *Mutat. Res*. **360**: 89–93

Vineger, R., Truax, J. F., Selph, J. L., Johnston, P. R. (1979) Antagonism of pain and hyperalgesia. Anti-inflammatory drugs. In: Vane, J. R., Ferreira, S. H. (eds) *Handbook of experimental pharmacology*. Vol. 50/II, Springer Verlag, Berlin, pp 208–222

Ward, D. T. (2004) Calcium receptor-mediated intracellular signalling. *Cell Calcium* **35**: 217–228

Wasicky, R. (1945) Anais da Faculdade de Farmácia e Odontologia da Universidade de São Paulo, vol. 4, São Paulo, p. 179

Wurdack, J. J., Smith, L. B. (1971) *Flora ilustrada catarinese: Polygalaceae*. Itajai, SC, Brazil, p. 28.

Zhang, D., Miyase, T., Kuroyanagi, M., Umehara, K., Noguchi, H. (1998) Polygala saponins from roots of *Polygala glomerata*. *Phytochemistry* **47**: 459–466

Zimmermann, M. (1983) Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* **16**: 109–110